Number/
Numéro

1

ISBN - 0 -7743-8797-1

PROCEEDINGS

TECHNOLOGY TRANSFER CONFERENCE No.4

PART 1

GENERAL RESEARCH

CONSTELLATION HOTEL , TORONTO

NOVEMBER 29&30 , 1983

SPONSORED BY

THE RESEARCH ADVISORY COMMITTEE

COORDINATED BY

RESEARCH COORDINATION OFFICE
POLICY & PLANNING BRANCH
MINISTRY OF THE ENVIRONMENT

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Program Committee: E. Piche
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G. Van Volkenburgh

Organization: D. Corr
M. Moselhy
C. Pautler

Administration: B. Malcolm

Mailing Address:

POLICY AND PLANNING BRANCH
Research Coordination Office
135 St. Clair Avenue West
Toronto, Ontario
M4V 1P5

Acknowledgements

The Ministry of the Environment's Research Advisory Committee would like to acknowledge the cooperation and efforts of the authors and Ministry staff who contributed to the organization of the Conference.

The financial support of the Provincial Lottery Trust Fund for environmental health-related research in Ontario has largely contributed to the success of this program including its technology transfer.

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PART 1

GENERAL RESEARCH

The Feasibility of the Sterile Male Approach
To
Control The Onion Maggot

F.L. McEwen, G.W. Ritcey and J. Madder

Department of Environmental Biology, University of Guelph,
Guelph, Ontario, N1G 2W1

INTRODUCTION

The sterile insect release method (SIRM) has provided effective control of a variety of severe pests, eg., primary screw-worm fly (Cochliomyia hominovorax) and mediterranean fruit fly (Ceratitis capitata). The use of this method results in a significant reduction in environmental contamination with insecticides and a reduction in residues in produce. The SIRM, while costly, has been economically viable when used against insects which are difficult to control with the more classical techniques (physical, cultural and chemical control methods).

At present, the only effective means of controlling the onion maggot (Delia antiqua) in commercial fields is with the intensive use of insecticides. This species has shown a remarkable ability to develop resistance to insecticides. In order to compensate for this resistance, growers increase the application rate until the maximum rate is used or until phytotoxicity is evident. Increasing application rates only delays the time when a new insecticide must be found to replace the old. In Ontario, the onion maggot has developed resistance to specific cyclodiene, carbamate and organophosphate insecticides with any one insecticide being effective for only a few years. This "pesticide treadmill" has been ongoing for more than 20 years with the onion maggot. The treadmill will abruptly end when no new effective insecticides are available. This point is being rapidly approached as few new insecticides are being registered, especially on minor crops. As a result, an effective alternative means of control must be available.

This project was designed to determine if the SIRM was biologically possible and economically viable when used for the control of the onion maggot. During 1981 and 1982, the project focused on the development of: a) mass rearing techniques; b) release methods; and c) field evaluation techniques.

In 1983, these techniques were used to mass rear onion maggots for a release in July. This report summarizes the work of the past three years.

MASS REARING TECHNIQUES

Adult Flies

Two types of cages were used for the maintenance of adult flies. Twenty cages (48 x 48 x 59 cm) were constructed of plexiglass with an access port on one side and an opening to allow air movement on the opposite side. The access port was closed with a cotton sleeve and the air opening covered with nylon screen. The cages were designed to make maximum use of the environment chamber in Graham Hall. These cages were relatively large, awkward and expensive to build. Therefore, the 214 cages used in the environment chamber in the Biological Control Laboratory were constructed with 1.2 x 1.2 cm spruce and nylon screen. Each (44 x 32 x 49 cm) cage was accessed through a 12 x 12 cm entry port, closed with a cotton sleeve. The sleeve and entry port of each type of cage could be removed to allow free access for initiation or cleaning.

Each cage contained two watering devices. Each device was made of a clay pot (9 cm diam.) with a rubber stopper in the drain hole. The pot was filled with distilled water, and the top covered with a plastic petrie dish. The dish was secured to the pot with an elastic band and the device inverted. The water percolated through the clay pot from which the adult flies could obtain water. Two devices in each cage provided water for ca. two weeks. Solid protein (Table 1) was provided in petrie dishes as required. The best diet was identified as described in Appendix 1. A carbohydrate souce was provided as required by applying a mixture of honey, sucrose and yeast hydrolysate (Table 1) to the top of the cage. The yeast hydrolysate was added to thicken the mixture and prevent its dripping into the cage.

Two plastic containers, each containing 3,250 pupae mixed with moist vermiculite, were placed into each cage. The moist vermiculite was added to prevent desiccation of the pupae. The containers with pupae were placed into the cages one day prior to the commencement of emergence. Eighty percent of the pupae emerged within four days of the start of emergence. Pupae reared for the colony had a mean emergence rate of 62 ± 10%. With this emergence success, ca. 4,500 adult flies were present in each cage after completion of emergence. This provided an absolute density of one fly/13.8 cm^3 in the plexiglass cages and one fly/19.4 cm^3 in the screen cages. These densities were used as they are in the optimum range for the space requirements for the onion maggot.

One week after the cage had been established, four oviposition dishes were placed into the cages with one in each corner. The adults frequent the corners of the cage and greater oviposition occurred if the dishes were placed in these areas. Each dish contained half an onion, with the cut-face partially buried in moist sand. This was found to be the most practical oviposition attractant as outlined in Appendix 2. The oviposition dishes were sprayed with water each day to maintain a high moisture level. This increased oviposition activity and decreased mortality of the first-instar larvae. The dishes were left in the cages for 72 h. By the end of this period some hatching had occurred and the first-instar larvae had entered the onion.

The adult flies were maintained for seven oviposition periods (21 days). Following this time, the watering devices and food dishes were removed and cleaned. The dead and surviving flies were removed, the cages washed and prepared for the next colony.

To ensure constant egg production, the 234 cages were divided into three groups of 78 cages each. Each group was started 12 to 14 days after the previous group. This provided a one-week pre-oviposition period, a three-week oviposition period and one week of free time for cleaning. The average egg production using this system was 2.4×10^6 eggs/week.

Larval Rearing Techniques

The onions used to feed the larvae were cut in half on their vertical axis with an automatic onion slicer. Cutting the onion on its vertical axis exposed the area preferred for feeding to the first-instar larvae, i.e., the meristemmatic tissue. In addition, more onions could be placed in each box when they were sliced in half.

Larvae were reared in corrugated cardboard boxes (46 x 60 x 15 cm) lined with a polyethylene sheet. A layer of moist vermiculite (2 cm deep) was placed over the liner. The onion halves were placed into the boxes with their roots down so that when the first-instar larvae migrated downward after hatching, they encountered an area of meristemmatic tissue. An average of 8.9 kg of onions were placed in each box.

To establish the mean oviposition rate per dish on a given day, oviposition dishes were randomly selected, removed from the cages and the eggs removed by flotation. These eggs were washed into a calibrated cylinder and the average number of eggs per dish was measured. The eggs and first-instar larvae in the oviposition dishes were washed with the sand and onion onto the top of the onion-filled box. Approximately 20,000 eggs were placed in each box as determined from the measured subsamples. A layer of moist vermiculite was spread over the eggs and onions so that the onions were completely covered. Seven L of vermiculite was used in each box. The box was placed onto rearing carts with 18 boxes/cart, and the cart moved into an incubator maintained at 23°C, photoperiod 8:16 L/D. The boxes were left in the incubator for 24 h during which time the eggs hatched and the first-instar larvae had the opportunity to migrate to the meristemmatic region of the onions. The box was left at 23°C if it was to produce nondiapause pupae. The nondiapause pupae could be used in the colony or released if insufficient time was available to terminate diapause. If the box was to produce diapause pupae, it was moved to an incubator maintained at 11°C, photoperiod 0:24 L/D. Larvae were reared under these conditions, as larval survival was much improved, i.e., $\bar{x} = 47\%$ at 11°C vs. $\bar{x} = 28\%$ at 23°C, and diapause pupae could be stored more successfully.

When raised under diapause conditions, the larvae required re-feeding approximately 40 days after hatching. However, as the number of eggs placed into each box was only approximate, the rate at which the food was used varied significantly. To identify when each box was to be re-fed, onion halves were placed on the top of the vermiculite 35 days after hatching. These onions were checked each day and when larvae were found in these onions, the boxes were re-fed. Re-feeding required 7 kg onion halves and 4 L of dry vermiculite. The vermiculite was added to reduce the amount of free water in the box. This reduced mortality due to the production of anoxic conditions and reduced drowning. When re-feeding, the new onions were placed below those that had decomposed. A second re-feeding was required for larvae reared under diapause conditions approximately 15 days later. The same procedure was followed as that of the first re-feeding, however 3 kg of onion halves were added and 1 to 3 L of vermiculite used to help dry the boxes.

Larvae reared under nondiapause conditions were re-fed once, 10 days after hatching. The same procedure was used as described above for the first re-feed of the diapause group.

Pupal Harvest Technique

Sixty days after the initiation of diapause boxes or 21 days after initiation of nondiapause boxes, the pupae were harvested. The entire contents of the boxes were dumped into a 0.8 m diameter drum constructed of 2 x 2 cm steel mesh. The contents of three boxes could be accommodated in the drum at one time. After loading, the steel mesh door was closed, secured, and the drum rotated on a horizontal axis. An electric motor was used to drive the drum at approximately 30 rpm for one minute. As the vermiculite and decomposed onions tumbled in the drum, the decomposed onions were broken into relatively small pieces. This allowed all of the pupae, vermiculite and small pieces of onion to fall through the mesh into a bin below the drum. As the drum was rotated, dry vermiculite was added so as to reduce the moisture content of the mixture.

The contents of the bin was shovelled onto the loading bins of two identical pupal separators. The separators consisted of a series of three horizontal screens aligned directly below each other. Each (54 x 180 cm) screen was constructed from a wood frame and steel mesh. The mesh sizes for the top, middle, and bottom screens were 8.0, 6.0 and 4.0 mm, respectively. The screens were held in a metal frame which provided support yet allowed the screens to move on a horizontal axis. The frame was attached to a camshaft driven by an electric motor which provided a horizontal shaking motion to the metal frame and screens. Each screen was sloped so that it had a drop of 7 cm over its length. Debris which could not pass through a screen was shaken down to its lower end and into collecting bins.

The vermiculite/pupae/onion debris mixture was gradually fed onto the top screen from the loading bin. The top screen stopped all debris larger than the pupae. The debris was collected, mixed with dry vermiculite and passed through the separator again to ensure the removal of all pupae. The pupae and vermiculite passed through the first screen and onto the second and third screens. These two screens retained all of the pupae but allowed the vermiculite to pass through. The vermiculite collected under the separator and was removed when necessary. When new larval rearing boxes were needed, the used vermiculite was sterilized and reused.

Flotation was used to remove organic debris and small lumps of vermiculite from the pupae. The pupae and debris were placed into a 50 x 50 x 30 cm metal tub which contained water to a depth of approximately 20 cm. The pupae were allowed to float to the surface and the debris settled to the bottom. The settled material was stirred to release any pupae from the debris. The pupae were removed from the surface of the water with a sieve, dried and, if necessary, cleaned with a stream of air. The pupae and debris were dropped through a stream of air which blew debris of lighter or heavier density away from the pupae. The extraction efficiency of the major stages of the pupal harvest is shown in Table 2.

Pupal Storage

The above extraction procedure produced clean, dry pupae with very little debris. If significant debris was present during storage, fungal growth could occur and reduce pupal vitality. The pupae were stored in one-L plastic containers sealed with a plastic lid with 15,000 pupae/container. Both diapause and nondiapause pupae were held at 4°C until needed, nondiapause pupae for the colony or release, diapause pupae for release. The diapause pupae had to be stored a minimum of 60 days to fulfill the requirements of diapause development.

Nondiapause pupae were removed from storage 11 days prior to the initiation of new adult cages. The pupae were removed from their storage containers and split into four groups of approximately 3,250 pupae. The pupae were mixed with moist vermiculite, placed into 0.5-L containers, and held at 23°C. One day prior to the commencement of emergence, the new cages were initiated and the containers with pupae were placed into the cages.

STERILIZATION AND RELEASE METHODS

In 1982 and 1983, diapause pupae were removed from storage and placed at 23°C for 10 days. The containers containing pupae were placed into a Gamma Cell and exposed to 3 krad of γ radiation. A 60-second exposure was required with two containers irradiated at one time. This timing and rate of irradiation was identified as the optimum, as described in Appendix 3. Both males and females were sterilized, however vitality was not affected.

In 1982, the irradiated pupae were mixed with moist vermiculite containing a red dye, placed into cages and allowed to emerge. The dye adhered to the ptilinum which was retracted shortly after emergence. In this manner all released flies were marked and could be identified after recapture. The adult flies were provided food and water as previously described. Thus the flies had the opportunity to feed and obtain moisture prior to the release, thereby increasing their ability to survive in the field.

Three days after the completion of emergence the cages were placed at 4°C, the flies removed from the cages and placed into one-L plastic containers. Each container held approximately 7,500 adult flies. Each container had two openings covered with nylon screen to allow for air movement. The containers were placed into coolers and maintained at 4°C until weather conditions were appropriate for the release; i.e., wind spread < 10 km/h and temperature $< 30^{\circ}$C. The flies did show significant mortality after 3 days of storage. When conditions were appropriate, the coolers containing an ice-pack and the pupae were transported to the Guelph airport. The flies were maintained at low temperatures to minimize their movement and respiration rate. They were loaded into the rear passenger compartment of a Cessna aircraft. Transportation to the airport required 20 min. and air-time to the release site required 30 min.

The flies were released over a 60-ha field from a height of approximately 50 m. The passenger removed the lid from the containers and poured the flies out of the passenger window. The aircraft was flown with 7° flaps so that the flies would not strike the plane after release. One million flies could be held and released on each flight. As this was a preliminary trial to test methodology, only one release of one million flies was made in 1982. This release was timed so as to correspond with emergence of the first portion of the second generation, i.e., 10 July in 1982.

In 1983, the irradiated pupae were mixed with moist sand and placed into 25 x 32 x 10 cm plastic tray. All of the flies released on 12 July 1983 were reared under diapause conditions and were marked with pink dye. Flies released on 21 July 1983 were marked with either green (nondiapause flies) or blue (diapause flies). The dyes were mixed into 2 cm of sand which was placed on top of the sand and pupae in the tray. As in 1982, when the flies emerged through the dye, it adhered to the ptilinum and allowed identification of each group of flies when they were recaptured. The plastic tray containing the pupae, sand and dye were placed on shelves in a picnic table enclosure made of nylon screen. This structure was used as a cage for the flies and was located in a controlled environment chamber maintained at 23°C. After emergence the adult flies were provided access of food (Table 1) for at least three days prior to each release.

On the date of release, the temperature in the environment chamber was lowered to 4°C minimizing the activity of the adult flies. All of the flies were removed from the cage by aspiration and placed into one-L plastic containers (the same as used in 1982). The containers were placed into 110-L plastic garbage pails along with ca. 9 kg of ice. The pails containing the adult flies were transported to the Keswick marsh in an airconditioned vehicle. The 3.0×10^6 flies in each release were distributed evenly throughout the release area between 19:00 h and 21:00 h. Five onion fields with a total area of 100 ha were used as the release area. Each of the five fields was divided into quadrants and an appropriate number of flies released in each quadrant so as to provide an even distribution of flies over the 100 ha. Previous trapping of adults had shown that the populations of onion maggots were similar in all five fields. The first release was timed by day-degree accumulations to coincide with the start of the second generation while the second release on 21 July was to augment and extend the effect of the first release.

FIELD EVALUATION

Fertility

In 1983, live flies were captured with interception traps two weeks before and two weeks following the release. In 1983, live flies were captured from three weeks before the first release to five weeks after the second release. Each trap was made of a metal frame 1.4 m high, 1.0 m wide and 0.3 m deep. The top and three sides were covered with nylon screen leaving one long side open. The adult flies would fly into the open side, encounter the screen and fly up to the top of the trap. Two glass jars were located at the top corners of the trap. Each jar contained a funnel which allowed the flies to enter, but not leave the jar. The traps were located at four locations, one set of traps on each of the four borders of the field. Two traps were placed at each location, one trap facing towards the crop and the other towards the hedgerow. In 1982, interception traps were placed around the treated field and around a 20-ha control field located 6 km away from the treated field. The same control field was used in 1983 and the treated (release) field was chosen at random from the 5 release fields. In both 1982 and 1983, the control field received insecticide treatments as deemed necessary by the grower, i.e., three treatments with diazinon during the second generation. The interception traps were checked 5 days a week. When checked, the bottles on the top of the traps were removed, sealed and replaced with empty jars. The sealed jars containing flies were returned to the field laboratory where they were anaesthetized with CO_2. The male flies were identified, their ptilinum everted and examined for dye. This process resulted in the death of the fly. The females were placed in a 910 cm^3 cage which was provided with food, moisture and an oviposition dish. Every two days thereafter the oviposition dish was removed and replaced with a new dish. The eggs were removed from the oviposition dish by flotation. They were placed into a petri dish (5 cm diam.) which was sealed and held at 23°C for three days. The proportion of the eggs hatching was determined by counting the total number of eggs and the number hatched three days after oviposition. After fertility was checked, the female flies were killed and the ptilinum everted and checked for dye. From previous laboratory studies, it was ascertained that sterilized released flies did not produce eggs. Therefore, all eggs layed were from the wild population.

Population Monitoring

Populations of adult flies in the treated and control fields were monitored using sticky traps. These traps were placed in the onion fields as early as possible after seeding and checked throughout the summer until the crop was windrowed. Each trap consisted of three wooden stakes (2.5 x 5.0 x 121 cm) placed in a row with 0.3 m between stakes. One-L milk cartons were painted yellow, coated with Tangletrap and placed into a notch on each stake. The cartons were kept just above the onion canopy to maintain trapping efficiency. The cartons were replaced at 10- to 15-day intervals or more frequently if they became covered with dust or large numbers of insects. The number of traps per field was dependent on field size. In 1982, the 60-ha treated field had 16 traps while the 20-ha field had 4 traps. In 1983, each field was trapped as follows: 40 ha - 8 traps, 2-20 ha fields - 8 traps, 15 ha field - 6 traps, 4 ha field - 4 traps. Each trap was randomly positioned along the sides of the field. The traps were examined two or three times each week throughout the growing season. Traps were checked at approximately the same time each day to prevent errors in fly counts caused by periodicity of flight activity. When the traps were checked, the flies were removed with a scraper and placed on a piece of bristol board. The flies were returned to the lab and their ptilinum checked for dye. In this manner the total number of flies caught and the proportion marked was determined.

The proportion of marked to unmarked flies caught in the interception and sticky traps provided the data to calculate a Lincoln Index.

Crop Damage

Crop damage was assessed in the treated and control fields using plots containing 100 onions. Each plot consisted of four double rows with 25 plants per row. Wooden stakes (25 cm x 5 cm) were placed in each row to denote the 100 onions in the plot. A red flag attached to a 1-m flexible rod was placed at each plot so that they could be found after the crop had grown. Ten plots were randomly placed in the field. The damage plots were examined after the first and second generations to determine the number of plants damaged by onion maggots. After the second generation the damage plots were removed. Damage to the crop at harvest, i.e., late second- and early third-generation damage was assessed when the onions were in windrows immediately prior to harvest. Ten samples of 100 onions each were examined for damage. The samples were taken near where the damage plots were located. Damage in the control and treated fields was compared after each generation and found not to be significantly different ($P \geq 0.05$).

The above studies (fertility, population size and crop damage) were found to be adequate to determine the efficacy of the sterile insect release method in comparison to normal grower practices.

SUMMARY

The techniques have been developed and tested for the mass rearing, sterilization, release and field evaluation of the sterile insect release method for control of the onion maggot. These techniques were used in 1983

for a large release against the second generation of the onion maggot in the Keswick Marsh. Six million flies were released resulting in a reduced fertility of ca. 35% for 3.5 weeks in the release area. (A complete report of the data is not possible at this time as it has not been fully analyzed). These data indicate that the use of the SIRM technique is a possible alternate control technique for the onion maggot. However, due to the relatively high cost of this method it is unlikely that it can be used on a large scale.

Table 1. Diets used for the maintenance of adult onion maggots.

"Protein Diet"	
	50% yeast hydrolysate
	50% Brewer's yeast
"Honey Diet"	
	80% honey
	13% sucrose
	7% yeast hydrolysate

Table 2. Number of pupae not extracted from the larval rearing media.

	Pupae Not Extracted	Percent Not Extracted
Drum	1,109	2.2
Shaker	3,126	6.3
Total	4,235	8.8

(Total number extracted 50,000)

APPENDIX 1. Effects of Diet and Cage Location on Oviposition of *Delia antiqua*.

Object: To determine the effect of diet and cage location on viable egg production by *Delia antiqua*.

Materials & Methods: The cages were established as follows:

- 16 screen cages
- 4 oviposition sites/cage
- 2 watering devices/cage
- 7,500 diapause pupae/cage
- free access to honey at the top of each cage
- free access to the specified diet

Diets used:

1. Hydrolysate Diet
   - 50% yeast hydrolysate
   - 50% Brewer's yeast
2. London Diet
   - 50% Brewer's yeast
   - 33% yeast hydrolysate
   - 17% soya flour
3. Guelph Diet
   - 37.5% soya flour
   - 25.0% sucrose
   - 12.5% Brewer's yeast
   - 12.5% yeast hydrolysate
   - 12.5% soya protein
4. Ticheler - Dutch Diet
   - 45% milk powder
   - 45% sucrose
   - 5% Brewer's yeast
   - 5% soya peptone

- The number of dead flies was counted before the first and after each 2-day oviposition period so that the number of viable eggs/living female could be determined for each period.
- A 4 x 4 latin square design was used with four diets assigned to each row and column of cages. Each diet was represented once in each column and row.

- Oviposition dishes were placed into cages For 13 periods with two days per period. The number of eggs oviposited in each cage, each period, was determined volumetrically.

- Fifty eggs from each cage were placed into petri dishes and checked for hatching five days later.

- The hypothesis was listed as a three-factor ANOVA with 13 replicates.

Results: Diet did affect egg production as shown below:

Diet	Viable Eggs/Female/Period*
1. Hydrolysate	3.58^a
2. London	3.11^a
3. Guelph	2.53^b
4. Dutch	1.43^c

---

*n = 52; means followed by different letters are significantly different ($P \leq 0.05$).

-There was no significant effect ($P \leq 0.05$) of row or column location on egg production.

Conclusions: The London and Hydrolysate diets were the best.

- Cage location had no significant effect on egg production.

APPENDIX 2. The Effect of Oviposition Attractants on Egg Production by *Delia antiqua*.

Object: To determine if specific natural, synthetic chemicals or fresh onion halves would enhance egg production by *Delia antiqua*.

Materials & Methods: The cages were established as follows:

- 16 screen cages
- 2 watering devices/cage
- 7,500 diapause pupae/cage
- free access to the Hydrolysate diet and honey
- 4 oviposition sites/cage; one in each corner

- The oviposition site consisted of a 9 cm diam. x 25 mm deep plastic petri cish. Each dish contained 50 ml of washed #24 silica sand and an attractant. After the attractant was added, 1/2 of a 6 cm diam. onion was partially buried in the sand with the cut face down.

- The chemical treatments were:

  1. 2.5 ml of 10% propanethol in methanol.
  2. 2.5 ml of 1% propyl disulfide in methanol.
  3. Rotting onions, obtained from the larval rearing boxes, were homogenized, the resulting fluid filtered and 2.5 ml of this fluid applied to the sand for Treatment 3.
  4. The control, which received only methanol.

- All of the treatments were moistened with a total of 12.5 ml water.

- One of each of the four treatments was placed in each of the 16 cages. One treatment was placed in each corner. The dishes were removed every two days and replaced with an appropriate treatment. The orientation of the treatments in the cages was based on a latin square design which was rotated. This allowed any one treatment to occupy one corner only once.

- Eight periods were run for a total of 16 days. This provided data for two latin square designs.

- After the dishes were removed from the cages, the eggs were removed by flotation and counted.

- These data were analysed using a three-factor ANOVA as a vested design. Egg deposition/period was the unit of measure.

Results: A significant difference was evident among the oviposition attractants as shown below.

Attractant	Average Number of Eggs/Period*
1. propanethol	143.8^a
2. propyl disulfide	124.1^a
3. onion rot	48.3^c
4. control	96.6^b

---

*n = 128; means followed by different letters are significantly different ($P \leq 0.05$).

Conclusions: The first and second treatments were more attractive than the half onion alone or onion and onion rot. However, due to the severe smell of these attractants and the relatively small difference as compared to the control. The control treatment was chosen for use in the colony.

APPENDIX 3. Sterilization of Delia antiqua.

Object: To identify the optimum rate and timing of irradiation of pupae of Delia antiqua in order to produce sterile adults.

Materials & Methods: Approximately 10,000 pupae were removed from storage and divided into two groups of equal size; one group to be iradicated, the other for a control.

- The pupae destined for irradiation were divided into six groups of equal size.

- All pupae were mixed with moist vermiculite and maintained at room temperature in one-L plastic containers.

- Nine and 12 days after removal from storage, one container with pupae was irradiated at each of 3,4 & 5 krad of irradiation.

- As irradiated flies emerged, they were separated on the basis of time of irradiation in relation to emergence (i.e., 2, 4, 6 & 8 days prior to eclosion) and by sex.

- The flies were placed into 910 cm^3 cages for crosses with flies from the control group. These cages contained food, a water source and an oviposition site.

- The sex ratio in all crosses was maintained at 1:1 with the number of flies per cross varying from 2 to 25. This was dependent upon the number of flies available on a specific day for each cross.

- The oviposition sites were removed every second day, the eggs separated by flotation and visibly counted.

- Egg fertility was established by holding the eggs for 5 days and counting the number of eggs which hatched.

- The spermatheca of males were removed, placed into a "wet mount", squashed and the presence or absence of motile sperm noted.

Results: There were no significant differences among the crosses resulting from pupae of different age (i.e., the time when the pupae were irradiated did not affect the results).

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Object: To identify the optimum rate and timing of irradiation of pupae of *Delia antiqua* in order to produce sterile adults.

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- The pupae destined for irradiation were divided into six groups of equal size.
- All pupae were mixed with moist vermiculite and maintained at room temperature in one-L plastic containers.
- Nine and 12 days after removal from storage, one container with pupae was irradiated at each of 3,4 & 5 krad of irradiation.
- As irradiated flies emerged, they were separated on the basis of time of irradiation in relation to emergence (i.e., 2, 4, 6 & 8 days prior to eclosion) and by sex.
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Results: There were no significant differences among the crosses resulting from pupae of different age (i.e., the time when the pupae were irradiated did not affect the results).

Table 1. Results from the crosses of sterilized females and fertile control males.

Radiation Dose (krad)	No. of Females Tested	No. of Eggs Laid	No. of Eggs Hatching	Percent Viability	No. of Eggs/Female
3	70	1	0	0	0.01
4	117	0	0	0	0
5	90	24	1	4.2	0.3

Table 2. Results from the crosses of sterilized males and fertile (control) females.

Radiation Dose (krad)	No. of Females Tested	No. of Eggs Laid	No. of Eggs Hatching	Percent Viability	No. of Eggs/Female
3	92	1,077	0	0	11.7
4	102	1,407	4	0.3	13.8
5	72	1,218	2	0.2	16.9

- The above tables indicate no significant difference among the 3 radiation doses. All resulted in very low viable egg production.

- Egg production of sterilized females was much reduced in comparison to control of females.

Table 3. Reproductive ability of control and sterile crosses.

Cross	No.of Females	No. of Eggs Laid	No. of Eggs Hatched	Percent Viability	No. of Eggs/Female	Insemination Rate (%)
♀ (virgin)	75	323	0	0	4.3	-
♂ x ♀	104	2,121	1,058	49.9	20.4	51.9
♂ x **♀**	277	25	1	4.0	0.1	47.4
♂ x ♀	266	3,702	6	0.2	13.9	34.0

♀ control female ♂ control male
♀ sterile female **♂** sterile female

- Untreated virgin females will lay eggs.

- The "control" cross (fertile male x fertile female) produced 20.4 eggs/female with an insemination of 51.9%.

- Sterile females have a much reduced egg production of 0.1 eggs/female but a similar incidence of insemination.

- Control females inseminated by sterile males laid eggs at a rate pf 13.9 eggs/female but these had less than 1% hatch.

- The insemination rate of these females was similar to but lower than the control.

- All observations concerning competitive ability and longevity showed no noticeable difference between treated and control flies.

Conclusions: Any treatment of 3 krad or more of any of the pupae listed produced effective sterility of both male and female onion maggots. The vitality of the treated flies was not reduced.

DEVELOPMENT OF METHODS FOR THE DETECTION OF ROTAVIRUSES

F.W. Doane[1], J.F.A. Hopley[1], P. Lavigne[1], and G. Jenkins[2]

[1]Department of Microbiology, Faculty of Medicine,
University of Toronto, Toronto, Ontario.
[2]Laboratory Services Branch, Ontario Ministry of the Environment,
Rexdale, Ontario

Rotavirus is the major etiological agent of infantile gastroenteritis, and is associated with up to 50% of the hospitalized cases of diarrheal illness in infants and young children. Between 1972-1979 there were 26 fatal rotavirus infections recorded at Toronto's Hospital for Sick Children. Because large quantities of virus are shed in the stool during infection, and because of the hardy nature of the virus, it is not surprising that rotavirus pollution of public water has become a matter of considerable concern. Sensitive virus detection methods are essential for monitoring the distribution of rotavirus in the environment. It has been the objective of this project to develop laboratory methods for the detection of rotavirus, and to design these methods so that they could be applicable to environmental samples.

In the initial phase of the project, simian rotavirus SA-11 was used as the virus model, and methods were investigated for detecting small quantities of rotavirus that had been isolated in cell cultures. Two new light microscope methods were developed: (1) the SPA method employing fixed *Staphylococcus aureus* cells as morphological markers; (2) a fluorescent antibody method (LAB) utilizing the high affinity of avidin for biotin. Both methods were compared with the more commonly used indirect immunofluorescence method (IIF).

The LAB method was found to offer the greatest sensitivity. It could detect, after 24 hours incubation, intracellular multiplication of an inoculum as low as 100 $TCID_{50}$, whereas the minimum inoculum size detectable by SPA was 125, and by IIF was 170. When approximately 20,000 $TCID_{50}$ of rotavirus was inoculated into cell cultures, its presence could be detected at 4 hours by LAB, 1 hour earlier than by the other two methods.

Current procedures for detecting viruses in the environment rely heavily on cell culture isolation. But by this approach there is danger of excluding several important viruses which do not grow readily in cell culture. Best known of these fastidious viruses are hepatitis A virus, Norwalk virus, certain strains of adenovirus, and human rotavirus. The next phase of this project should concern itself with the application of methods such as the LAB method, as well as immunoelectron microscopy and enzyme-linked immunosorbent assay (ELISA), for *direct* detection of viruses in environmental samples, thus bypassing the extremely useful but highly selective cell culture isolation step.

INTRODUCTION

The present study has been part of a long-term project to develop sensitive and reliable methods for detecting viruses that might pollute drinking and recreational water. The specific objective of the present study has been to develop a method for detecting rotaviruses.

Rotaviruses constitute one of the most serious causes of gastrointestinal infections among infants and young children. Besides causing a great deal of morbidity, rotaviruses may also cause fatal disease. Between 1972 and 1979, 24 fatal cases of rotavirus were documented at the Hospital for Sick Children (Middleton 1982).

Diarrhoea is the main clinical feature of rotavirus infections, and large quantities of virus are shed in the stools. Being non-enveloped, rotaviruses are relatively hardy viruses, consequently they can remain infectious for long periods of time, especially when adsorbed to particulate material.

Despite the fact that rotaviruses are responsible for such a large proportion of gastrointestinal infections, especially among infants and neonates, laboratory studies on these viruses have been slow in coming. Rotavirus is one of the "newer" viruses, and it was not discovered, by laboratory methods, until 1973 (Bishop et al., 1973; Flewett et al., 1973). This long delay in detecting a virus that causes such a high incidence of morbidity has been due to the fact that human rotavirus cannot be grown by the usual tissue culture methods employed routinely for other viral pathogens.

To date there has been only limited success in growing human rotaviruses in tissue culture, and this has required such methods as preliminary passage of the virus in animals (Wyatt et al, 1976; 1980), and centrifugation of the inoculum onto susceptible cell monolayers (Banatvala et al., 1975; Bryden et al., 1977). Animal strains of rotavirus have been less fastidious, consequently studies on rotaviruses and rotavirus infections have dealt primarily with non-human strains, such as Nebraska calf diarrhoea virus (NCDV), and simian rotavirus SA-11. Both of these viruses grow well in cell culture. Furthermore - and of particular importance - certain viral antigens are common to all species of rotaviruses, consequently detection of human rotavirus antigens and antibiodies can be carried out using e.g. NCDV or SA-11 reference strains.

OBJECTIVES

The immediate objective of this study has been to develop a sensitive and reliable method for rotavirus detection that would be applicable to environmental samples. A variety of laboratory methods are currently in use for detecting rotavirus in clinical specimens (faeces), and these include:

A. Direct examination of the specimen for the presence of virus particles or viral antigens by -
   (i) electron microscopy (EM)
   (ii) enzyme-linked immunosorbent assay (ELISA)
   (iii) counterimmuno electrophoresis (CIOP)

B. Inoculation of the specimen into cell cultures in order to amplify the virus content (applicable only to rotavirus strains that can be grown in cell culture), then detection and identification of virus by -

(i) electron microscopy

(ii) ELISA

(iii) fluorescent antibody technique (immunofluorescence)

The approach to the immediate objective has been as follows:

STAGE 1. Using NCDV and SA-11 develop a method that will give improved detection sensitivity relative to existing standard methods.

STAGE 2. Apply this method to the detection of rotaviruses in environmental samples (rather than clinical specimens).

STAGE 3. Develop methods for the isolation, detection and growth of human rotavirus strains.

REPORT OF STAGE ONE OF ROTAVIRUS PROJECT

Materials and Methods

Viruses

NCDV rotavirus was obtained from Dr. F. Bishai, Central Laboratory of the Ontario Public Health Laboratories. It was maintained by passage in BSC-1 cell cultures. To prepare virus pools, TC flask cultures of BSC-1 cells were washed twice with phosphate buffered saline (PBS), and inoculated with a dilution of virus calculated to give a 3+ CPE within 24 hr. Virus was allowed to adsorb for 1 hr. at room temperature. Maintenance medium consisting of MEM + HEPES buffer + 5 μg trypsin/ml + 0.5 - 0.75% bovine serum albumen (BSA) was added, and flasks were incubated at 37°C. At 3+ CPE, the cells were frozen and thawed 3 times, centrifuged at 1100 rpm for 10 min, and the supernatant removed and used as the stock virus pool (stored at-70°C).

SA-11 virus was obtained from Dr. L. Spence, Toronto General Hospital, and was grown in MA-104 cell cultures. To prepare virus pools, TC flask cultures of MA-104 cells were washed 3 times with PBS and inoculated with 1-2 ml of SA-11 virus that had been treated for 20 min with 10 μg/ml of trypsin. Virus was allowed to adsorb for 1 hr at 37°C before the addition of a serum-free nutrient medium consisting of Eagle's minimum essential medium (MEM) prepared with HEPES buffer, supplemented with 5 μg/ml of trypsin and 0.75% bovine serum albumin (BSA). Cells were incubated until a 3+ CPE was observed. They were then taken through three cycles of freezing and thawing, the cell lysate was centrifuged at 1100 rpm for 10 min, and the supernatant was removed and used as the stock virus pool (stored at - 70°C).

Purified virus pools were prepared by cesium chloride isopyric centrifugation, according to the method of Petric et al. (1975).

Cell Cultures

MA-104 cells (established fetal rhesus monkey kidney cells) were obtained from Dr. P.J. Middleton, Hospital for Sick Children. Cultures were prepared in 75 or 150 cc plastic flasks in a growth medium consisting of supplemented Eagle's minimum essential medium (MEM) in HEPES buffer, plus 5% fetal calf serum, and gentamycin. Two days after trypsination the cells were changed to maintenance medium, which was the same as the propagating medium except for a reduction to 2% fetal calf serum.

Both stationary and roller tube coverslip cultures of MA-104 cells were used. Lab-Tek tissue culture chambers (Miles Laboratories, Rexdale, Ontario) were incubated in a moist atmosphere at 37°C, in the presence of 5% CO_2. Glass coverslips, approximately 3 x 15 mm in size, were inserted in rubber-stoppered tissue culture tubes. Coverslip cultures were rotated in a roller drum at 1/3 rpm at 37°C for 2 days, a procedure that has been shown to enhance rotavirus infectivity (Sato et al., 1981, 1982). Cultures were then ready for virus inoculation.

BSC-1 cells (established African green monkey kidney cells) were obtained from Mr. Goff Jenkins, Ontario Ministry of the Environment. They were grown in MEM + HEPES buffer, supplemented with 5-10% calf serum, 10% tryptose phosphate broth, and gentamycin. Maintenance medium contained 2-5% calf serum.

Electron Microscopy

Samples to be examined by electron microscopy were processed by the agar diffusion negative staining technique (Anderson and Doane, 1972) as follows: a 25 μl drop was placed on a plastic-coated EM specimen grid resting on 1% agar. When the drop had dried (45-60 min) the grid was removed, exposed briefly to 2% phosphotungstic acid pH 7 (negative stain), air-dried, and examined in a Philips EM 300 transmission electron microscope.

Indirect Immunofluorescence (IIF) Method

Coverslip cultures were washed with PBS, and inoculated with 0.1 ml of SA-11 rotavirus stock. Virus was allowed to adsorb at 4°C for 1-2 hr. At a predetermined time the fluid was withdrawn from the culture tube, the cells were washed three times with PBS, and fixed at -20°C for 10 min in absolute ethanol. The pH was adjusted by rinsing in PBS. The coverslip was then removed from the tube and placed, face up, on moistened filter paper in a petri dish. Rabbit anti-NCDV hyperimmune antiserum (obtained from Dr. L. Spence) was layered on top of the culture (25 μl of antiserum) and incubated at 37°C for 40 min. The culture was washed at least three times in PBS, then layered with 0.1 ml of goat anti-rabbit immunoglobulin antiserum that had been conjugated to fluorescein isothiocyanate (FITC) (Antibodies Inc., Calif.). After 40 min at 37°C the coverslip was rinsed in PBS, dried in air, mounted on a glass slide in fluorescent antibody medium pH 7.2 (Difco Laboratories, Detroit, Mich.), and sealed with nail polish. The mounted preparation was examined in a Zeiss incident-light fluorescence microscope equipped with a filter combination 48 77 09 + in the blue - ultraviolet excitation range 450-490 nm.

Experimental Procedures and Results

A. Development of a Protein A Method for Rotavirus Detection

The IIF immunoassay is a standard method that is used universally for detection of viral antigens (Gardner & McQuillin 1980). It is generally employed in the indirect form, which requires a primary anti-viral antiserum and a secondary anti-species antiserum that has been conjugated to FITC. It was our intention to replace the secondary reagent with a preparation of Staph. aureus cells, which have a unique protein (A) on the bacterial cell wall that has a strong affinity for immunoglobulins of many animal species (Mallinson et al., 1976). Protein A in purified form has been used extensively in immunoassays; however, in this study we elected to use the intact (fixed) bacterial cell, employing the bacterium itself as a morphological marker or label at the light microscope level (Goding 1978; Lancz & Specter 1982; Morgenson & Dishon; Surolia et al., 1982).

A variety of experiments were carried out, to investigate the suitability of the SPA method for rotavirus detection, and to compare the Staph. aureus Protein A (SPA) method with the indirect immunofluorescence test.

1. Preparation of Staphylococcus aureus Protein A. The Cowan strain of Staph. aureus ATCC 25904 was obtained from Dr. S. Vas, Toronto Western Hospital. Four colonies grown on blood agar plates were transferred to 5 ml of Todd-Hewitt broth and grown overnight at 37°C. This was then added to 100 ml of Todd-Hewitt broth and incubated for 18 hr at 37°C. The resultant dense culture was dispensed into centrifugation tubes and spun at 3000 rpm for 20 min. The supernatant was removed and a whitish

pellet with a greyish ring remained. The pellet was resuspended in approximately 20 ml of PBS, and the last procedure was repeated twice. The final pellet was resuspended in 2% formaldehyde in PBS and left for 3 hr at room temperature. This suspension was spun down and the pellet washed twice in PBS then resuspended in 5 ml of PBS and placed in a water bath at 80°C for 10 min. After centrifugation the pellet was resuspended in 10% w/v of PBS containing 0.1% sodium azide. A sterility check was performed before storing the preparation at 4°C.

The concentration of bacterial cells was determined by counting the cells in a microchamber, using light microscopy.

2. Basic procedure for detecting rotavirus in cell culture, using the SPA method. Virus inoculation and subsequent treatment of coverslip cultures was performed essentially as described above for IIF. After exposure to antiviral antiserum, cultures were rinsed in PBS and exposed to an appropriate dilution of SPA at 37°C for $\geq$ 30 min. Cultures were then rinsed in PBS, stained for 5 min with the fluorochrome dye acridine orange (AO), according to the method of Goldner et al., 1983, rinsed in citrate - phosphate buffer and mounted.

Results. Control and uninfected cultures of MA-104 cells treated with SPA and stained with acridine orange exhibited orange cytoplasm, yellow-green nuclei and orange nucleoli. Infected cultures treated with SPA and stained with AO contained foci of cells covered with orange-staining bacterial cells. The cocci appeared to be confined entirely to areas over the cytoplasm, with none being seen on the surface over nuclei.

3. Establishing the specific activity of the SPA method. The specific activity of the SPA detection method was demonstrated by a series of controls. Results are given in Table 1. It can be seen that a positive result was obtained only in the presence of a combination of virus, viral antibody, and SPA.

4. Establishing the specific activity of the IIF method. The specific activity of the IIF method was also investigated, in order to assess more fully the advantages and disadvantages of the two rotavirus detection methods. In the IIF method, the presence of antigen is indicated by the presence of fluorescence; there should be little or no "background" (non-specific) fluorescence. As shown in Table 2, there was a strong background fluorescence in cell control and infected cultures stained with FITC.

5. Determination of optimum concentrations of antiserum and SPA. Chessboard titrations of varying concentrations of anti-NCDV antiserum and SPA were tested, in order to determine the optimal concentrations required to give maximum labelling of infected MA-104 cells. As shown in Table 3, when SPA cells were used at a concentration of 3×10^{10}, maximum labelling was achieved with antiserum dilutions $\leq 1/80$; if antiviral antiserum was diluted byeond 1/80, the distribution of label decreased. SPA cells at a concentration of 1.5×10^9 also produced maximum labelling, provided the antiviral antiserum was not diluted beyond 1/50. When SPA cell concentrations $< 1.5 \times 10^9$ were used, sub-optimal labelling was obtained.

At SPA cell concentrations $>1 \times 10^{10}$, non-specific clumping of bacterial cells occurred; this was not a problem when SPA cell concentrations were < 2×10^9. Consequently, the optimum SPA concentration to use was estimated to be approximately 1.5×10^9 cells/ml.

6. Determination of optimum incubation times for antiserum and SPA. It was found that, to obtain maximum SPA labelling, it was necessary to incubate infected cells in the presence of antiviral antiserum for $\geq$ 40 min (Table 4). The SPA incubation period required to produce a positive reading was $\geq$ 20 min, with maximum labelling occurring when both antiserum and SPA were incubated for 40 min or more. On the basis of these experiments it was estimated that antiserum and SPA incubation periods should each be 60 min.

7. Comparison of SPA sensitivity versus other rotavirus detection methods. The SPA detection method was compared with several other methods to determine their relative sensitivity in detecting the presence of rotavirus. Two separate experiments were performed. The first involved the inoculation of known amounts of SA-11 virus into MA-104 cell cultures; after 24 hr the cultures were examined for rotavirus by each of three light microscope methods - cytopathic effect (CPE), IIF and SPA. It can be seen from Table 5 that the detection end point for all three methods was 10^{-6}. However, of greater importance was the observation that when an inoculum dilution $\leq 10^{-3}$ was used, both the IIF and SPA methods were more sensitive than CPE in detecting the presence of virus, with SPA being slightly more sensitive than IIF. When one calculates, by the Kärber method, the virus titre obtained by each method, there is almost a 2 log improvement in sensitivity in favour of the immunoassays.

In the second experiment carried out to compare the sensitivities of the different methods, a determination was made of the minimum time required, post-inoculation, for virus to be detected. As shown in Fig. 1, virus was first detected at 6 hr by both IIF and SPA. In contrast, a clear CPE was not evident until approximately 15 hr.

B. Enhancement of the Fluorescein Signal in the IIF Method

Technical problems with the IIF method were encountered in the earlier stages of this project, including extensive non-specific background fluorescence, and fading of the fluorescence during the long photographic exposure times required for the weak signal. Thus, although the IIF method proved to be almost as sensitive as SPA for rotavirus detection, the FITC signal was much more difficult to record, both visually and photographically. The purpose of this phase of the project was to attempt to overcome some of these problems by increasing the signal to noise ratio and amplifying and stabilizing the FITC signal.

It was found that background fluorescence could be reduced by incorporating 1% bovine serum albumen (BSA) in the PBS washes employed immediately pre-and post-fixation, prior to the addition of antiviral antiserum. The background fluorescence could be reduced even further by counter staining with 0.0001% Congo red in PBS, for 5 min at 22^{0}C, prior to mounting (Gardner and McQuillin, 1980).

The problem of fading of fluorescence during photographic exposure was eliminated by using the chemical antibleach para-phenylanediamine (Johnson et al., 1981, 1982) incorporated in a mounting medium of glycerol and Moviol (Hoechst Canada Inc., Montreal, P.Q.). It was also found that by using Kodak Ektachrome daylight film of ASA 200 or ASA 400, rather than tungsten film of ASA 160, the exposure time for IIF could be reduced. In combination, these factors improved considerably the usefulness of the IIF method.

C. Development of a Labelled Avidin-Biotin (LAB) Immunoassay

The LAB immunoassay has been introduced only recently into diagnostic virology, and has the potential of being one of the most sensitive of those methods used to detect antigens. Avidin is a 68,000 molecular weight glycoprotein from egg white which has an extremely high affinity for biotin (vitamin H or coenzyme R) (Heitzmann & Richards, 1974; Green 1975; Bayer & Wilchek 1980), the avidin-biotin association constant being 10^{-15} M (compared to 10^{-5} to 10^{-9} M for antigen-antibody associations). Biotin can be covalently coupled to free amino groups on a protein such as IgG, and avidin can be conjugated to light microscope labels such as horseradish peroxidase (Guesdon et al., 1979) or FITC (Berman & Basch, 1980). Label amplification occurs as a result of the presence of four biotin binding sites on the avidin molecule (Green 1975). The 3-stage LAB assay used in our study (Figure 2) involved (1) an initial antigen-antibody interaction, (2) the addition of an anti-species antibody conjugated to biotin, and (3) the addition of an avidin-FITC conjugate.

LAB procedure. Coverslip cultures were washed twice in 1% BSA in PBS for a total of 10 min. Cells were fixed for 5 min in acetone, rinsed in 1% BSA in PBS, and coverslips were transferred to petri dishes containing moistened filter paper. A volume of 25 µl of antiviral antiserum (rabbit) was placed on each coverslip, which was then incubated at 37°C for 40 min.

After a brief PBS wash, cultures were exposed to 25 μl of antispecies antibody (goat anti-rabbit) conjugated to biotin (Cappel Laboratories, West Chester, P.A.) for 30 min at 37°C, washed again in PBS, incubated at 37°C for 30 min in the presence of avidin-FITC conjugate (Miles Laboratories, Elkhart, Ind.), washed 3 x in PBS, counterstained with 0.001% Congo red for 5 min, rinsed in PBS, air-dried thoroughly, and finally mounted in Moviol-glycerol-antibleach medium. Appropriate controls, comparable to those used for IIF and SPA, were used to ensure the specific activity of the LAB assay. Microscopy and photomicrography procedures were as described above for IIF.

Results. MA-104 cells infected for 18 hr with SA-11 virus and processed by the LAB method contained cells with discrete, bright areas of fluorescence scattered throughout the cytoplasm. In control and in infected cultures exposed to LAB, there was a uniform distribution of non-specific, faint background fluorescence (Table 6). However, its appearance was in marked contrast to the discrete fluorescing areas of viral antigen, and the two could easily be differentiated. Results of a chessboard titration carried out to determine the optimum concentrations of the conjugates that would give the best signal: noise ratio are given in Table 7. From these results it was determined that the optimum dilution of biotin-conjugated antiserum was 1/1500 and that of avidin-FITC was 1/50.

D. Comparison of IIf, SPA and LAB Methods for Rotavirus Detection

At this final stage of the first portion of the project, the three immunoassays - IIF, SPA and LAB - had been raised to maximum efficiency for detecting rotavirus antigen in cell culture. It was now necessary to determine which of the three methods would be most suitable as a routine detection method. The following aspects of each method were investigated: (1) the earliest detection of antigen in infected cells; (2) the minimum amount of virus inoculum that could be detected 24 hours post inoculation (p.i.); (3) the minimum amount of antiviral antiserum required for a positive reaction; (4) the quality of the fluorescent signal, as recorded visually and photographically; (5) the cost and level of difficulty of setting up the test.

Results. When MA-104 cell cultures were inoculated with a multiplicity of infection of 1:25 (2×10^4 $TCID_{50}$/0.1 ml), viral antigen was first detected 4 hr p.i., by the LAB method only. One hour later, at 5 hr p.i., antigen was detected by all three methods (Table 8). The minimum inoculum that could be detected 24 hr p.i. was approximately 100 $TCID_{50}$, and this was detected only by the LAB method. The minimum inoculum required for IIF was approximately 125 $TCID_{50}$, and was approximately 175 $TCID_{50}$ for SPA. The LAB method required the smallest amount of antiviral antiserum, and could be used at almost twice the dilution required for SPA, and 1 1/2 times less than that needed for IIF.

DISCUSSION AND RECOMMENDATIONS

In a recent study group report published by the International Association for Water Polution Research (1983), the following recommendations were made:

"Virological research concerned with the optomization of monitoring systems and quality limits should include the improvement of techniques for the recovery and detection of small numbers of viruses in large volumes of water. Special attention should obviously be given to gastroenteritis viruses such as Norwalk- and rotaviruses, and the hepatitis A virus. These techniques are necessary to study the incidence and behaviour of viruses in water, and to establish relations between viruses and indicators, which are essential to develop reliable surveillance systems and quality limits which may not include virus tests." It has been the objective of the study reported here to develop such methodology for rotavirus detection. Because of our rather extensive experience with the simian rotavirus SA-11 (Quan 1982; Quan and Doane, 1983) we have used this virus in MA-104 cells as our model system throughout the first phase of the study. The standard cell culture detection procedures used as reference methods against which we compared our methodology were (a) cytopathic effect (CPE) and (b) indirect immunofluorescence (IIF).

IIF is undoubtedly one of the most important immunoassays used in diagnostic virology (Gardner and McQuillin 1980). However, IIF requires a great deal of "fine-tuning" to eliminate problems such as non-specific or background fluorescence in order to ensure reliable specificity coincident with high sensitivity. We were able to reduce background fluorescence by the introduction of two steps: (1) the use of a 1% bovine serum albumin - PBS wash prior to the addition of FITC label, and (2) the use of the counterstain Congo Red.

A second problem we encountered with IIF was the fading of the FITC label during the long exposures required for photomicrography. During the early stages of the project, it was virtually impossible to record the fluorescent label, photographically, during a 2 min exposure, due to photobleaching. Subsequently, by using a mounting medium consisting of Moviol mixed with glycerol and the chemical antibleach para-phenylenediamine (Johnson et al., 1981, 1982), the problem of fluorescence fading was eliminated.

Despite the improvements made to the IIF method, its sensitivity in detecting rotavirus was never greater than the SPA or LAB methods, both of which had certain technical advantages over IIF. The SPA method, which makes use of the unique affinity of Staphylococcus aureus Protein A for the Fc-region of immunoglobulins, is relatively inexpensive to prepare. The label is the bacterium itself, which can easily be prepared in any microbiology laboratory. The label is detected by acridine orange staining of the preparation, in combination with ultraviolet or short wavelength blue radiation. Under these conditions the bacterium appears, by light microscopy, as a distinct orange coccus (Goldner et al., 1983). When the SPA method is used to detect SA-11 virus infection in MA-104 cells, the bacterial label attaches to any antiviral antibody that has complexed with intracellular viral antigen. As in the case of the IIF method, it is necessary first to solublize the plasma membrane with acetone to permit exposure of the antigen to the surface and to enhance passage of the viral antibody to the antigen.

The SPA method was almost as sensitive as the LAB method in detecting rotavirus antigen; after an incubation period of 24 hours it could detect an inoculum of 125 $TCID_{50}$ versus 100 $TCID_{50}$ detected by the LAB method. Furthermore, it was more sensitive than IIF, which could detect only 170 $TCID_{50}$ at 24 hours. Finally, microscopy and photomicrography with SPA were both superior to the other methods, due to the distinctive label and the intensity of the acridine orange stain.

We introduced the labelled avidin-biotin (LAB) method because of its impressive association constant of 10^{-15} M; this is many orders higher than that recorded for antigen-antibody (10^{-5} - 10^{-9}M). Only very recently have avidin and biotin immunoassay reagents been available commercially, but the few publications describing their use report increased sensitivity and decreased reagent requirements. In our study on rotavirus we obtained similar results. By LAB we detected the smallest amount of antigen. When a multiplicity of only 1 $TCID_{50}$ of virus per 25 cells was inoculated into cell cultures, progeny viral antigen could be detected as early as 4 hours p.i. This is particularly impressive when one considers that the earliest detection of rotavirus by electron microscopy, using the same virus-cell system, has been found to be 6 hours (Quan & Doane, 1983).

Conclusions. On the basis of the results obtained from stage 1 of this project, we recommend the use of the LAB method for detecting rotavirus antigen in cell culture. Extending this further, we recommend its use for the rapid detection of any virus that has been isolated in cell culture.

However, like other immunoassays it has one major limitation. Because of the high degree of specificity of the viral antisera used in immunoassays, each virus must be tested for on an individual basis, and heterologous antigens will go undetected. For this reason, we conclude that it is still more appropriate,in general, to identify by electron microscopy viruses isolated in cell culture from environmental samples. As was shown in an earlier study (Doane et al., 1982) a wide range of common viral pathogens can be detected by immunoelectron microscopy (IEM) using human immune serum globulin. Their type identification can then be carried out by IEM, using intersecting antiserum pools.

Future recommendations. On the basis of the results reported here, we recommend that the LAB method now be applied to direct rotavirus detection in environmental samples themselves. Although cell culture isolation procedures currently being used are excellent for amplifying the concentration of many viruses, including animal rotavirus, they will miss human rotavirus, as well as other fastidious viruses such as hepatitis A virus, Norwalk virus, and certain pathogenic strains of adenovirus. Thus the objective should be to reduce the dependancy on cell culture isolation, and to develop methods for direct detection. Two detection methods that should be applied at an early stage are the LAB method, and an ELISA method incorporating avidin and biotin.

As has already been noted, a major factor in favour of using cell cultures is the virus amplification achieved. This is an important consideration, as large quantities of virus are essential if one is to produce reference antisera by immunization of animals, or if epidemiological surveys are to be carried out on the distribution and transmission of a particular virus. In view of the importance of the human rotavirus as a cause of morbidity and mortality, we consider it to be a top priority to continue with the studies initiated during the past year for the growth of human rotavirus *in vitro*. Preliminary results are encouraging, and show that at least some strains of the human virus can be induced to grow *in vitro*.

We further recommend that attention be directed to the detection of Norwalk virus. It has been estimated that approximately 1/3 of epidemics of gastroenteritis are produced by Norwalk virus or closely related viruses (Wolf & Schreiber, 1982). The agent has not yet been propogated definitively *in vitro*, and is detected in feces by electron microscopy (Kapikian et al., 1972, 1979). Because of the absence of any reference antisera, it will be necessary to rely, initially, entirely on electron microscopy. However, we anticipate that the use of immune serum globulin in combination with electron microscopy will offer a sensitive detection method.

Investigation of methodology for hepatitis A virus is hampered by the high level containment restrictions required for work on this virus. Until such time as the necessary facilities are readily available, it will be difficult to conduct research in this area.

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TABLE 1. Controls Used to Assess the Specific Activity of the Rotavirus - SPA Method

Controls (AO-stained)	MA104 Cells Uninfected	 Infected
Cells alone	-*	-
Cells + normal rabbit serum (NRS)	-	-
Cells + anti-NCDV antiserum	-	-
Cells + SPA	-	-
Cells + NRS + SPA	-	-
Cells + pooled human γγ + SPA	-	+
Cells + anti-NCDV antiserum + SPA	-	+

* Presence (+) or absence (-) of bacterial cells on cell cultures. SA-11 virus was used at a concentration of 2×10^4 $TCID_{50}$/ml. Infected cultures were examined at 18 hr. post inoculation.

TABLE 2. Controls Used to Assess the Specific Activity of the Rotavirus - IIF Method

Controls	MA104 Cells Uninfected	 Infected
Cells alone	- *	-
Cells + NRS	-	-
Cells + anti-NCDV antiserum	-	-
Cells + anti-Ig-FITC	- (bf)	- (bf)
Cells + NRS + anti-Ig-FITC	- (bf)	- (bf)
Cells + pooled human γγ + anti-Ig-FITC	- (bf)	- (bf)
Cells + anti-NCDV + anti-Ig-FITC	- (bf)	+ (bf)

* Presence (+) or absence (-) of specific fluorescence.
bf = background fluorescence.
SA11 virus was used at a concentration of 2×10^4 $TCID_{50}$/ml.
Infected cultures were examined at 18 hr. post inoculation.

TABLE 3. Chessboard Titration to Determine the Optimum Concentrations of Rotavirus Antiserum and SPA Cells.

Antiserum Dilution (Reciprocal)	SPA concentration (cells/ml)							
	$3x10^{10}$	$1.5x10^{9}$	$4x10^{7}$	$2x10^{7}$	$1.3x10^{7}$	$1x10^{7}$	$8x10^{6}$	$4x10^{6}$
1	4+*	4+	2+	1+	1+	1+	1+	1+
10	4+	4+	2+	1+	1+	1+	1+	1+
20	4+	4+	2+	1+	1+	1+	1+	1+
40	4+	4+	2+	1+	1+	1+	1+	1+
50	4+	4+	2+	1+	1+	1+	1+	1+
80	4+	2+	1+	1+	0	0	0	0
100	2+	2+	1+	1+	0	0	0	0
200	1+	1+	1+	0	0	0	0	0

* Extent of SPA label recorded on rotavirus-infected cell culture (4+= max.) SPA volume used = 0.1 ml/culture.

TABLE 4. Chessboard Titration to Determine Optimum Incubation Times for Rotavirus Antiserum and SPA Cells.

Antiserum Incubation Time (Minutes) at 37°C	SPA Incubation Time (Minutes) at 37°C							
	5	10	20	30	40	60	80	120
10	0*	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
30	0	0	1+	1+	1+	1+	1+	1+
40	0	0	1+	4+	4+	4+	4+	4+
60	0	0	1+	4+	4+	4+	4+	4+

*Extent of SPA label recorded on rotavirus-infected cell culture (4+= max.). SPA volume used = 0.1 ml/culture.

TABLE 5. Comparison of Sensitivity in Detection of Rotavirus of Three Different Methods

Dilution of Rotavirus Inoculum	Rotavirus Detection Method		
	CPE	IIF	SPA
10^{-1}	15/15*	5/5	5/5
10^{-2}	15/15	5/5	5/5
10^{-3}	6/15	5/5	5/5
10^{-4}	3/15	4/5	5/5
10^{-5}	3/15	3/5	3/5
10^{-6}	3/15	2/5	3/5
10^{-7}	0/15	0/5	0/5
10^{-8}	0/15	0/5	0/5

Calculation of $TCID_{50}$/ml by Kàrber (log $TCID_{50} = D - \Delta\ (S - 0.5)$):

CPE	IIF	SPA
~ 3.2×10^{4}	~ 2×10^{6}	~ 2×10^{6}

*Proportion of inoculated cultures recorded as positive.

TABLE 6. Controls Used to Assess the Specific Activity of the Rotavirus - LAB Method

Controls	MA104 Cells	
	Uninfected	Infected
Cells + mountant	-*	-
Cells + buffer	-	-
Cells + anti-NCDV antiserum	-	-
Cells + B-Ab	-	-
Cells + Av-FITC	-	-
Cells + anti-NCDV + B-Ab	-	-
Cells + anti-NCDV + Av-FITC	-	-
Cells + B-Ab + Av-FITC	- (bf)	- (bf)
Cells + NRS + B-Ab + Av-FITC	- (bf)	- (bf)
Cells + anti-NCDV + B - Ab + Av-FITC	- (bf)	+ (bf)

* Presence (+) or absence (-) of specific fluorescence.
bf = background fluorescence
B-Ab = biotin-conjugated antibody
Av-FITC = FITC - conjugated avidin
NRS = normal rabbit serum

TABLE 7. Determination of Optimum Concentrations of Biotin-Conjugated Antibody and FITC-Conjugated Avidin for the LAB Rotavirus Method.

		Dilution of FITC-Avidin (reciprocal)				
		1	10	100	1000	2000
Dilution of Biotin-Conjugated Antibody (reciprocal)	4	10/1*	10/1	10/1	10/1	3/.3
	32	10/1	10/1	10/1	10/1	3/.3
	256	10/1	10/1	10/1	10/1	3/.3
	512	10/1	10/1	10/1	10/1	3/.3
	1024	10/1	10/1	10/.6	6/.6	3/.3
	2048	10/1	10/.6	10/.6	0/0	0/0
	4096	0/.3	0/.3	0/.3	0/0	0/0

*S/N ratio (Maximum signal = 10; maximum noise = 1)

TABLE 8. Comparison of IIF, SPA and LAB Rotavirus Detection.

	IIF	SPA	LAB
Earliest detection of antigen p.i. (M.O.I. = 1:25; 2×10^4 $TCID_{50}$)	5 hr	5 hr	4 hr
Minimum virus inoculum required ($TCID_{50}$/0.1ml)	170	125	100
Minimum dilution of antiviral antiserum required	1/1260	1/1000	1/1600
Visual quality of signal	3+	4+	3+
Film exposure time required	1-2 min	10-15 sec	1-2 min
Cost	moderate	low	moderate

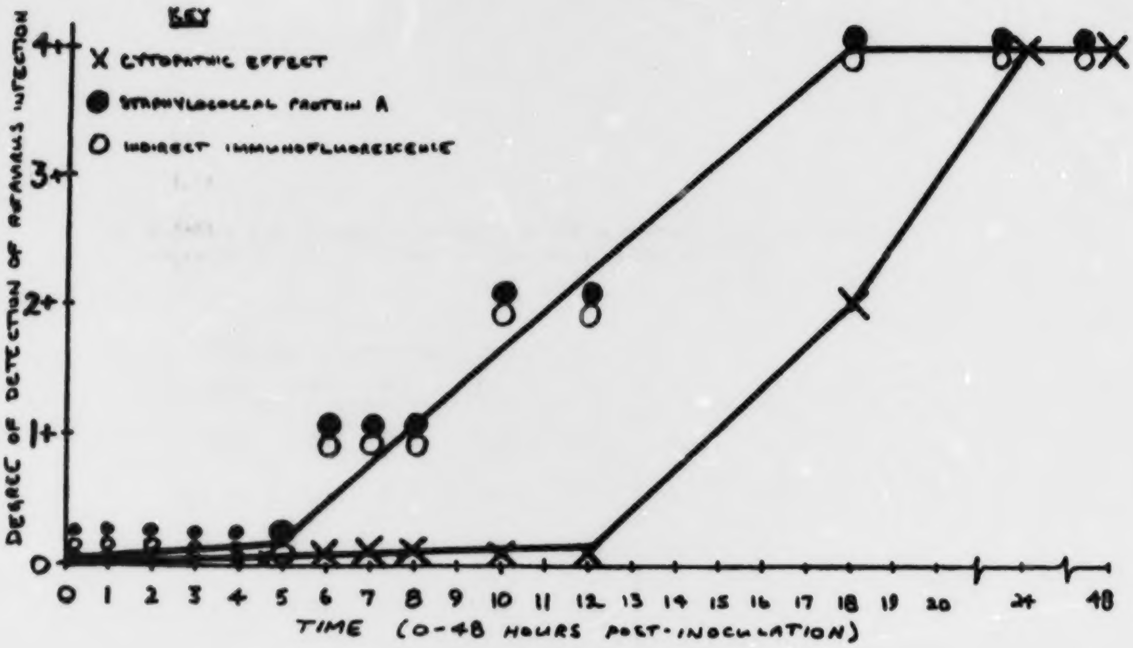

Figure 1. Comparison of the minimum time required for rotavirus detection by three different methods.

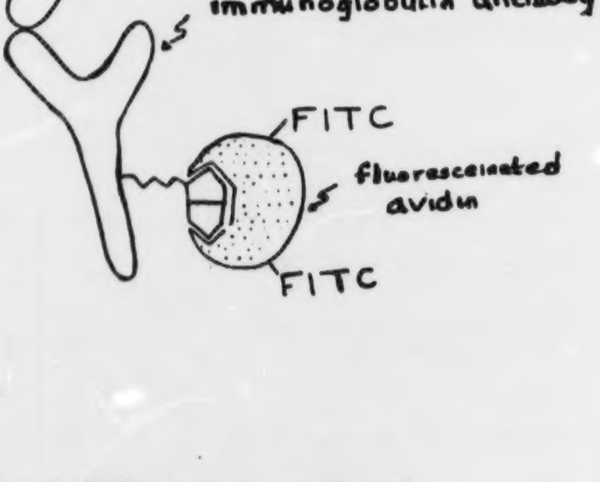
rotaviral antigen
antiviral antibody
biotin-conjugated anti-immunoglobulin antibody
FITC
fluoresceinated avidin
FITC

Figure 2. Reactants involved in the LAB method.

THE EPIDEMIOLOGY OF SWIMMING - RELATED ILLNESS AT SELECTED CONSERVATION AREAS

P.L. Seyfried and N.E. Brown
Department of Microbiology and Department of Community Health
University of Toronto, Toronto, Ontario. M5S 1A8

Abstract

During the summers of 1979, 1980, and 1982 swimmers and non-swimmers were interviewed in prospective cohort epidemiological - microbiological studies carried out at beaches in Ontario. Of more than 8,000 people interviewed, 9.4% of the swimmers became ill versus 3.4 to 3.7% of the non-swimmers. Swimmers experienced respiratory ailments most frequently followed by: gastrointestinal, eye, ear, skin, and allergic symptoms, respectively. Water samples collected at the beaches were routinely analyzed for fecal coliforms, fecal streptococci, total staphylococci, and viruses and, in most surveys, for heterotrophic bacteria and *Pseudomonas aeruginosa* organisms as well. In 1980, viruses were isolated from the water at two conservation area beaches but not from the water of any of the Great Lakes beaches surveyed. Morbidity among swimmers was shown to be related to staphylococcal counts ($p < 0.0004$) and fecal coliform levels ($p < 0.0058$). Data from a 1983 study which was conducted at Boyd, Claireville, Heart Lake, Kelso and Albion Conservation Areas and at Professors's Lake in Brampton will be used to augment the results from previous surveys.

Introduction

Swimming at public beaches is a very popular pastime in the province of Ontario. Akin *et al*. (1983) reported that the World Health Organization (1976) revealed that, annually, approximately 500 million people are affected by incapacitating water-borne or water-associated illnesses, and that about 25% of the world's hospital beds are occupied due to unwholesome water.

In Ontario, the guideline for fecal coliforms, the present recreational water quality indicator of choice in North America is a geometric mean density of 100 bacteria per 100 ml of water (Ontario Ministry of the Environment, 1978). The limit is 200 fecal coliforms per 100 ml of water in most other Canadian provinces and American states (Cabelli et al., 1979; U.S. Environmental Protection Agency, 1976). There is a dearth of epidemiological evidence, however, to support the current recreational water quality guidelines (Cabelli et al., 1979, 1976; Foster et al., 1971). Documentation of swimming-related illness is, however, existent (Brown, 1983; Baron et al., 1982; Cabelli et al., 1982; Koopman et al. 1982; Calderon and Mood, 1981; D'Alessio et al., 1981; Cabelli, 1980; Cabelli et al., 1979; Ktsanes et al., 1979; Seyfried and Fraser, 1978; Ortiz, 1977; Rosenberg et al., 1976; Cabelli et al., 1976, 1975; Bryan et al., 1974; Stevenson, 1953).

As a result of several prospective cohort epidemiological studies, Cabelli et al.(1982); Cabelli,(1980) detected measurable health affects (10/1000 swimmers experienced "highly credible" gastrointestinal symptoms), at Escherichia coli and enterococcal densities as low as 10 organisms per 100 ml of water, when only 10 to 50 ml of water was ingested. These investigators also promoted the enterococci as the optimal recreational water quality indicator.

In our 1980 prospective cohort epidemiological - microbiological survey, a total of 3,967 swimmers and 2,105 non-swimmers were interviewed at 10 beaches in Ontario. Lake water and sediment samples were collected at the beaches and analyzed for fecal coliforms, fecal streptococci, heterotrophic bacteria, Pseudomonas aeruginosa, and total staphylococci. Bacterial densities were found to be approximately 10 times higher in the sediment than in the corresponding surface water samples.

It was observed in the 1980 study that 77 (3.7%) of the non-swimmers became ill compared to 371 (9.4%) of the swimmers. Because morbidity among swimmers was shown to be related to staphylococcal counts ($p < 0.0004$) and fecal coliform levels ($p < 0.0058$), Brown (1983) has advocated the use of total staphylococci as a bacterial freshwater recreational water quality indicator.

This paper describes the 1982 component of the on-going investigations designed to detect and quantitate swimming-related morbidity, and to evaluate the use of fecal coliforms and total staphylococci, in particular, as bacterial recreational fresh-water quality indicators. In this particular study, limited virological analyses were performed in order to detect viruses in fresh recreational waters.

2. Materials and Methods

i) Epidemiology

A prospective cohort epidemiological investigation was undertaken, from July through August of 1982, at Boyd Conservation Area (north-west of Toronto, and south of Kleinburg) in Ontario. The investigation was modeled upon Cabelli's American studies (Cabelli et al., 1982; Cabelli, 1980; Cabelli et al., 1979, 1975).

More than 1300 persons were interviewed at Boyd Conservation Area beach, on weekends. Weekend sampling was used in order to obtain maximum numbers of beach-going subjects (Cabelli et al., 1982; Cabelli, 1980; Cabelli et al. 1975). A trained interviewer wearing a t-shirt bearing the words: "Health and Water Quality Study" attempted to interview as many beach-goers, as possible. Family units, in contrast to groups of friends, were preferentially selected, in order to facilitate accurate and accessible follow up information. Beach groups of size six or under were considered optimal. During the initial interview, a contact or spokesperson was appointed for each beach group. The telephone number, address, and best time of day to reach the contact person were recorded. In general, the mother of the family, if present, was selected as the contact person because of her knowledge of family morbidity. Subsequently, each member of the individual beach group was interviewed, at the beach.

A copy of the initial interview form (A1) employed at the beach is attached. Follow-up was done exclusively by telephone. A copy of the telephone follow-up form (A2) is also provided. Confidentiality of information was stressed throughout the study. Telephone follow-up was done within five to seven days after the initial interview, and the period three days after the initial interview day was followed for morbidity development. Three days was the critical interval chosen such that the information would be readily recallable, would not overlap with the next weekend's sampling, and so that illnesses with long term incubation periods would be excluded, due to the possibility of excessive, confounding variables, as well as attempting to avoid detection of person-to-person spread of disease.

ii) Computer Analysis

All the data were coded for record purposes. Data analyses were performed using SAS (Statistical Analysis System) (SAS Institute Inc., 1982). FUNCAT was the technique employed in SAS, to develop the models for total morbidity in swimmers. Basically the predictive model was, as follows, for swimmers:

$$\text{logit } p = \ln \frac{p}{1-p} \quad \frac{(\text{ ill })}{(\text{not ill})} = \mu + \alpha + \beta + \gamma \text{ (count)},$$

where μ is the mean of the illness rate or intercept for the model, α and β are binomial factors (of varying number) which significantly altered the model, and γ is the bacterial count (based on the 3 groups used for counts i.e.: 0-150, 151-500, > 500 organisms/100 ml).

iii) Bacteriological Analyses

Surface water samples were collected at Boyd beach, and occassionally at Claireville Conservation Area and Albion Hills Conservation Area (both north-west of Toronto), at a water depth of at least 50 cm, in a location with a high density of swimmers.

Water was analyzed for: total coliforms, fecal coliforms, fecal streptococci, and total staphylococci. All filtrations of appropriately diluted* samples were done using 0.45 μm Gelman filters.

Total coliforms were detected by placing filters upon M-Endo Agar LES (Difco) (51.0g/l to which 20 ml of 95% ethanol had been added). Incubation of plates was done at 35^{o}C for 22 hr $\pm$2 hr. The plates were incubated in a humid atmosphere. Colonies exhibiting green or gold metallic sheens were considered positive.

Fecal coliforms were detected by placing filters upon m-Tec Agar (see A3). The plates were put into a cakette with ice and incubated at 44.5^{o}C for 23$\pm$ 1 hr. Ice allows for the gradual rise of the incubation temperature to44.5^{o}C. After incubation plates were allowed to stand at room temperature for 30 minutes to allow development of the stronger colour reaction by Klebsiella sp. colonies. All yellow or predominently yellow(i.e. yellow-brown, yellow-green) colonies were considered positive.

Total staphylococci were detected by placing filters upon Vogel-Johnson Agar (Difo) with 0.5% sodium pyruvate. Plates were incubated at 35^{o}C for 48 hr. Round, black, shiny colonies were considered positive. Fecal streptococci were detected by placing filters upon m-Enterococcus Agar (Difco) (42.0 g/l). Plates were incubated at 35^{o}C for 48 hr. $\pm$ 3 hr. Red, maroon, or pink colonies were considered positive.

iv) Virological Analyses

Virological analysis was performed twice, due to budget constrictions. The methods are outlined in detail elsewhere (Jenkins and Cherwinsky, 1982). The method is a membrane filtration procedure using 40 l surface water samples. Final indentification was accomplished using electron microscopy.

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* Dependent on turbidity.

Results and Discussion

A list of the dates when epidemiological interviewing was carried out at Boyd Conservation Area is given in Table 1. Completed interviews totalled 1366, there were 107 refusals, and 45(3.2%) incompleted interviews due to such problems as wrong telephone number, no telephone, or long distance calls.

A 100% telephone response rate was achieved in the study, if one disregards interviews which were not possible to follow-up. This supports previous findings (Brown, 1983) that in studies of this type, telephone follow-up is preferable to follow-up by mail, or giving forms away at the beach that would subsequently be returned by mail.

In Table 2, the microbiological sampling dates are listed and the geometric means of the total coliform, fecal coliform, fecal streptococci and total staphylococci counts are given. The suggested guidelines for total coliforms, fecal coliforms, and total staphylococci are limits of 1000, 100, and 100, respectively, and a fecal coliform/fecal streptococci ratio of 4 or less (Ontario Ministry of the Environment, 1978; Brown, 1983). At Boyd Conservation Area, on either all sampling days or epidemiological sampling days, the limits were surpassed for fecal coliforms, and total staphylococci, but not for total coliforms (apart from epidemiological sampling days) and the fecal coliform/fecal streptococcal ratio . At Claireville, the limits were exceeded for total coliforms, fecal coliforms, and total staphylococci, but not for the fecal streptococcal ratio . At Albion Hills Conservation Area, none of the limits were surpassed, although sampling was done on only one day. Clearly, Boyd and Claireville display some evidence of bacterial recreational water pollution, (Claireville generally more than Boyd,) and as such should be monitored closely, for public health purposes.

Reovirus was detected at Boyd Conservation Area on July 25th, 1982 (Table 2). On that day, the total coliform, fecal coliform, and total staphylococcal limits were all exceeded, in contrast to the fecal coliform/fecal streptococcal ratio. Reovirus' role in clinical morbidity has not been clearly established (Jenkins and Cherwinsky, 1982). Virus went undetected when the total staphylococcal count (the only bacterial count done on August 2nd) was 10 or acceptable. Thus, on July 25th, 1982, total coliforms, fecal coliforms, and total staphylococcal counts reflected the virological problem, in some manner.

In this study there were 873 swimmers (64%) and 493 non-swimmers (36%) analyzed. A comparison of the types of illness experienced by the swimmers and non-swimmers is shown in Table 3. The p values provided are from Fisher's 2-tailed test. As may be seen from the table, the crude morbidity rates revealed a statistically significant difference in illness between swimmers and non-swimmers for total morbidity ($p = 0.0000$), gastrointestinal ailments ($p=0.0025$), and "other" ailments ($p=0.0260$), in contrast to ear, eye, respiratory, allergenic, and skin symptoms.

No direct relationship between variability in bacteriological counts and illness was apparent (Tables 2 and 4).

The data were adjusted for individual categories as shown in Table 5, however, no major alterations occurred. Nonetheless, it is necessary to consider the interactions of these factors, using models, to elucidate any effect that the factors might have on the data.

No evidence existed for a relationship between smoking cigarettes and elevation of respiratory morbidity in either swimmers or non-swimmers.

The predictive models for swimmers to become ill (total morbidity) are presented in Tables 6 and 7. For the fecal coliform model, the factors which significantly altered swimmers becoming ill were eating home food (protective) and putting one's head underwater (protective; this may reflect lack of accurate data from the contact person or splashing). The fecal coliform counts did not significantly contribute to the model (p=0.4573), however this may be a function of the limited scale of the study.

For the total staphylococcal model, the factors which significantly altered swimmers becoming ill were: eating beach food (unprotective; a risk factor), eating home food (protective), swallowing water (unprotective), and putting one's head underwater (protective-perhaps as explained above). Total staphylococcal counts did not significantly contribute to the model (p=0.3746) which may be a function of the limited scale of the project, however their probability for predicting illness is a little better than that for fecal coliforms, given the two models.

Summary

1. Boyd and Claireville Conservation Areas display some evidence of bacterial recreational water pollution, and thus should be monitored routinely.
2. Although sampling was limited, reovirus was detected in the water at Boyd Conservation Area.

3. Swimmers became ill (total morbidity) (9.4%) more than non-swimmers (3.4%) (significant with p = 0.0000 to 4 decimal places). There was also a statistically significant difference between swimmers and non-swimmers for gastrointestinal (p=0.0025) and other ailments (p=0.0260). These statements apply to crude morbidity rates.

4. Predictive models, although based on results from a small sample size, tended to indicate a better dose-response relationship for total staphylococci (at levels higher than 150 organisms/100 mL) than for fecal coliforms. We therefore recommend the use of both total staphylococci and fecal coliforms as bacterial recreational water quality indicators.

5. Future investigators should consider the roles of swallowing water, and eating beach and home foods in studies of this nature.

6. Future studies should investigate the possibility of establishing a viral recreational water quality guideline.

TABLE 1

BOYD CONSERVATION AREA EPIDEMIOLOGICAL INTERVIEWING DATES

Dates (n = 11 days)
3/7/82
4/7/82
10/7/82
11/7/82
24/7/82
25/7/82
31/7/82
1/8/82
7/8/82
14/8/82
15/8/82

No. of completed interviews = 1366

No. of refused interviews = 107

No. of incompleted interviews due to wrong telephone number, no telephone, or long distance calls = 45.

TABLE 2

i) Boyd Conservation Area Microbiological Data.

Date	Total Coliform Count (per 100 ml)	Fecal Coliform Count (per 100 ml)	Fecal Streptococci Count (per 100 ml)	Total Staphylococci Count (per 100 ml)	Virus Detected
*3/7	190	32	18	190	ND
*4/7	1055	224	102	1000	ND
*10/7	580	430	191	247	ND
*11/7	655	288	368	278	ND
17/7	890	185	110	460	ND
*24/7	1100	325	290	383	ND
*25/7	1233	607	280	623	+ (Reovirus)
*31/7	ND**	ND	ND	143	ND
*1/8	ND	ND	ND	937	ND
2/8	ND	ND	ND	10	-
*7/8	2067	584	257	353	ND
*14/8	4500	2433	533	337	ND
*15/8	2400	1030	780	1010	ND
22/8	330	90	100	72	ND
25/8	ND	ND	ND	623	ND
28/8	300	130	115	33	ND
29/8	100	10	10	72	ND
Overall mean	1185[a] (741)	490[a] (229)[a]	243 (148)	423[a] (240)[a]	ND
Mean for epidemiology days.	1531[a] (1096)[a]	661[a] (398)[a]	313 (219)	500[a] (407)[a]	ND

Bracketed values indicated geometric means.

* = Epidemiological sampling dates.

a = Exceeds limit.

** = Not done.

TABLE 2 (Continued)

ii) Claireville Conservation Area Microbiological Data

Date	Total Coliform Count (per 100 ml)	Fecal Coliform Count (per 100 ml)	Fecal Streptococci Count (per 100 ml)	Total Staphylococci Count (per 100 ml)
3/7	5000	2200	510	ND
4/7	ND	ND	ND	3000
10/7	2000	440	128	513
17/7	8950	520	270	633
24/7	7650	1445	260	367
25/7	4767	1533	830	420
31/7	ND	ND	ND	250
1/8	ND	ND	ND	683
7/8	3133	1000	487	700
14/8	3000	1247	760	427
15/8	2250	1440	910	540
22/8	ND	220	200	92
28/8	690	105	85	40
29/8	100	10	50	73
Overall mean	3754[a] (2344)[a]	924[a] (501)[a]	408 (288)	595[a] (347)[a]

Bracketed values indicate geometric means.

a = Exceeds limit.

TABLE 2 (Continued)

iii) Albion Conservation Area Microbiological Data

Date	Total Coliform Count (per 100 ml)	Fecal Coliform Count (per 100 ml)	Fecal Streptococci Count (per 100 ml)	Total Staphylococci Count (per 100 ml)
4/7	20	4	2	45

TABLE 3

CRUDE MORBIDITY RATES

(Symptom rates per 1,000 persons)*

Category of Illness	Number of Swimmers Ill	Number of Non-Swimmers Ill	Fischer's p value to 4 decimals (swimmers versus non-swimmers for actual numbers)
Total	(82/873) 94	(17/493) 34	0.0000**
Gastrointestinal	(23/873) 26	(2/493) 4	0.0025**
Other	(19/873) 22	(3/493) 6	0.0260**
Ear	(6/873) 7	(0/493) 0	0.0931
Eye	(5/873) 6	(0/493) 0	0.1662
Respiratory	(40/873) 46	(15/493) 30	0.1973
Allergy	(4/873) 5	(0/493) 0	0.3032
Skin	(2/873) 2	(0/493) 0	0.5384

Note: Apart from total morbidity, one person may appear in more than one category.

Where: Total illness = all ailments except sunburn
Gastrointestinal = stomachache, or diarrhea, or vomiting
Ear = earache or runny ears
Eye = styes, or red, itchy, or watery eyes
Respiratory = sore throat, or runny or stuffed nose, or fever, cold or cough
Allergy = allergenic itch, or welts or sneezing
Other = ailments other than above and sunburn

* Using rounded whole numbers. ** Significant at p = 0.05 or less

TABLE 4

CRUDE TOTAL MORBIDITY DATA BY DATE

(Boyd Conservation Area)

Date	Swimmers ill (%)	Non-swimmers ill (%)	% Difference
3/7	5/34 (14.7%)	3/71 (4.2%)	10.5%
4/7	8/59 (13.6%)	2/54 (3.7%)	9.9%
10/7	15/70 (21.4%)	1/22 (4.5%)	16.9%
11/7	4/84 (4.8%)	2/32 (6.3%)	-1.5%
24/7	7/100 (7%)	1/26 (3.8%)	3.2%
25/7	7/86 (8.1%)	0/42 (0%)	8.1%
31/7	5/63 (7.9%)	4/55 (7.3%)	0.6%
7/8	4/99 (4.0%)	1/39 (2.6%)	1.4%
10/8	9/94 (9.6%)	1/44 (2.3%)	7.3%
14/8	7/89 (7.9%)	2/58 (3.4%)	4.5%
15/8	11/95 (11.6%)	0/50 (0%)	11.6%

TABLE 5

ADJUSTED MORBIDITY RATES

Type of Illness and Adjustment	Symptom Rates per 1000 persons Swimmer N= 873	 Nonswimmer N= 493
All Illnesses		
Unadjusted	94	34
Sex	94	32
Age	92	38
Contact person	94	35
Swam before or after	93	45
Beach food	93	36
Home food	98	32
Socioeconomic status	91	35
Respiratory		
Unadjusted	46	30
Sex	46	28
Age	44	35
Contact person	46	31
Swam before or after	45	41
Beach food	46	32
Home food	49	27
Socioeconomic status	45	31
Cigarette smoker	46	30
Skin		
Unadjusted	2	0
Sex	2	0
Age	2	0
Contact person	2	0
Swam before or after	2	0
Beach food	2	0
Home food	3	0
Socioeconomic status	1	0

TABLE 5 (cont'd)

	Swimmer N= 873	Non-swimmer N= 493
Eye		
Unadjusted	6	0
Sex	6	0
Age	5	0
Contact person	6	0
Swam before or after	5	0
Beach food	6	0
Home food	6	0
Socioeconomic status	6	0
Gastrointestinal		
Unadjusted	26	4
Sex	26	4
Age	25	3
Contact person	27	4
Swam before or after	26	4
Beach food	26	4
Home food	26	4
Socioeconomic status	24	4
Ear		
Unadjusted	7	0
Sex	7	0
Age	7	0
Contact person	7	0
Swam before or after	7	0
Beach food	7	0
Home food	7	0
Socioeconomic status	7	0

TABLE 5 (cont'd)

	Swimmer	Nonswimmer
Allergy		
Unadjusted	5	0
Sex	5	0
Age	5	0
Contact person	5	0
Swam before or after	5	0
Beach food	4	0
Home food	4	0
Socioeconomic status	5	0
Other		
Unadjusted	22	6
Sex	22	6
Age	23	6
Contact person	22	6
Swam before or after	22	7
Beach food	22	7
Home food	23	7
Socioeconomic status	21	6

TABLE 6

PREDICTIVE MODEL FOR SWIMMERS TO BECOME ILL
(USING FECAL COLIFORMS) - BOYD CONSERVATION AREA

$$\text{logit } p = \ln \frac{p}{1-p} = -\ 1.6977 \text{ (intercept, or mean illness rate)}$$

(- 0.5377 (if ate home food)
(or + 0.5377 (if did not eat home food)

(- 0.2800 (if put head underwater)
(or + 0.2800 (if did not put head underwater)

(+ 0.2518 (if fecal coliforms = 0 - 150 per 100 ml. of water)
(or + 0.002804 (if fecal coliforms = 151 - 500 per 100 ml.)
(or - 0.2546 (if fecal coliforms = > 500 per 100 ml.)

1. p = ill.

   1-p is not ill.

2. This model is based upon total morbidity data.

3. In the model the effects of the factors involved are as follows:

Source	X^2 p value
intercept	0.0001
eating home food	0.0009
head underwater	0.0368
fecal coliform counts	0.4929
likelihood ratio	0.4573 (no significant lack of fit).

TABLE 7

PREDICTIVE MODEL FOR SWIMMERS TO BECOME ILL (USING TOTAL STAPHYLOCOCCI) - BOYD CONSERVATION AREA

logit p = $\frac{p}{1-p}$ = - 1.4959 (intercept, or mean illness rate)

(+ 0.3058 (if ate beachfood)
(or - 0.3058 (if did not eat beach food)

(- 0.5226 (if ate home food)
(or + 0.5226 (if did not eat homefood)

(+ 0.4362 (if swallowed water)
(or - 0.4362 (if did not swallow water)

(- 0.2667 (if put head under water)
(or + 0.2667 (if did not put head under water)

(- 0.1300 (if total staphylococci = 0 -150 per 100 ml. of water)
(or - 0.1038 (if total staphylococci = 151 - 500 per 100 ml.)
(or + 0.2338 (if total staphylococci = > 500 per 100 ml.)

1. p = ill. 1-p = not ill.
2. This model is based upon total morbidity data.
3. In the model, the effects of the factors involved are as follows:

source	X^2 p value	
intercept	0.0001	
eating beach food	0.0107	
eating home food	0.0009	
swallowing water	0.0150	
head underwater	0.0346	
total staphylococcal counts	0.3746	
likelihood ratio	0.2961	(no significant lack of fit).

A1. INITIAL INTERVIEW FORM

DATE: ☐☐ TIME: ☐:☐ I.D. ☐☐☐☐☐☐☐☐☐

LEGEND: YES - 1 NO - 0 DON'T KNOW - 3

I. GIVEN NAMES	1.	2.	3.	4.	5.	6.
II. RELATIONSHIP TO CONTACT PERSON						
III. AGE (Approx. if Adult) & SEX						
IV. SWAM IN PAST 4 DAYS? (Yes or No)						
WHERE AND WHEN?						
HEAD UNDER?						
V. SWAM, OR WILL SWIM TODAY?						
HEAD UNDER?						
VI. SMOKE CIGARETTES?						
under ½ pack daily?						
½ - 1 pack daily?						
more than 1 pack daily?						
VII. SMOKE PIPE? OR CIGAR?						
VIII.* CANCER IN FAMILY?						
TYPE?						
WHAT RELATION?						
XI. CONTACT PERSON ONLY:**						
No. persons in household?						
No. rooms in household?						

PHYSICAL SYMPTOMS ("C" for current;
"P" for past week)

1. COLD, UPPER RESPIRATORY						
2. EAR PROBLEMS						
3. STYES OR BOILS						
4. GASTROINTESTINAL						
5. ALLERGIES						
6. SUNBURN						
7. OTHER (NO. & NOTE)						

PERSON'S FULL NAME			
ADDRESS			NOTES
POSTAL CODE			
PHONE NO.			
BEST TIME TO CALL			

* Omitted, eventually due to general distaste expressed

** Due to time factor involved

A2. TELEPHONE FOLLOWUP FORM

DATE: ☐☐ I.D. ☐☐☐☐☐☐☐☐☐

LEGEND: 1 - YES 0 - NO

NAME:

I. Swam on contact day?						
Head under?						

<table>
<tr><td>II. Swam in next 3 days?</td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td></tr>
<tr><td>Which days? (Circle)</td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td></tr>
<tr><td>At this beach only? (Yes or No)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Head under? (Yes or No)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

III. Eye goggles?						
Ear plugs?						
Nose plugs?						
Swallow any water?						
Eat food bought at beach? (or drink)						
Eat food, at beach, brought from home? (or drink)						

PHYSICAL SYMPTOMS

(Write in date when first noticed)

A. Sore throat						
B. Fever						
C. Cold or cough						
D. Runny or stuffed nose						
E. Earache, Runny Ears						
F. Styes or red, itchy or watery eyes						
G. Stomach ache or nausea						
H. Diarrhea						
I. Vomiting						
J. Boils						
K. Skin rash						
L. Allergenic itch, welts or sneezing						
M. Sunburn: Mild						
N. Moderate						
O. Severe						
P. Other symptoms						
Q. Did you see doctor?						
R. What was his diagnosis?						
S. Did you stay at home because of illness?						
T. How many days?						

If illness serious, may we have your doctor's name and phone number?

A3 m-TEC Agar Formulation

Proteose peptone #3 (Difco)	5.0 g
Yeast extract (Difco)	3.0 g
Lactose (Difco)	10.0 g
NaCl	7.5 g
K_2HPO_4 (BDH)	3.3 g
KH_2PO_4 (BDH)	1.0 g
Sodium lauryl sulphate (Difco)	0.2 g
Sodium desoxycholate (Difco)	0.1 g
Bromocresol purple (BUH)	0.08 g
Bromophenol red (MCB)	0.08 g
Agar (Difco)	15.0 g
Distilled water	1000 ml

- heat to boiling to dissolve agar
- autoclave: 15 mins. at 121°C
- cool to 50°C and dispense in square plates

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THE REMOVAL OF TRIHALOMETHANE PRECURSORS AND SYNTHETIC ORGANIC CHEMICALS FROM POTABLE WATER SUPPLIES BY COAGULATION.

F. Tonelli, H. Donison, A. Benedek, Zenon Environmental Inc.,
845 Harrington Court, Burlington, Ontario L7N 3P3

ABSTRACT

Coagulation is widely used in potable water treatment for the removal of turbidity causing particles. This study investigated the potential of the coagulation process for organic removal, both natural and synthetic. A wide variety of conventional coagulants, recently developed pre-polymerized coagulants and flocculants were studied, with the aid of jar tests.

Results indicate that coagulants are generally highly effective in removing natural organics. Synthetic organics on the other hand do not appear to be removed by coagulation.

As much as 80% of the natural organics present in the water can be removed by coagulation. Consequently, trihalomethane formation, due to chlorination, can be substantially reduced. The operating pH was found to have a significant effect on the removal of organics and also the formation of trihalomethanes. Maximum organic removal typically occurred in the pH range of 5.0 to 6.0. The lowest trihalomethane formation per unit weight of TOC typically occurred at pH 6.0 or below. However, for the aluminum based coagulants, the optimum pH was found to be between 6.0 and 7.0. Above and below these pH's residual aluminum in the water often exceeded the MOE guideline of 0.1 mg/L.

Results of jar tests and filtrability tests showed that the pre-polymerized coagulants both settled and filtered more readily.

INTRODUCTION

In recent years, the removal of organic compounds from potable water supplies has become a matter of great concern to water treatment engineers. Potable waters contain natural organics (compounds such as humic and fulvic acids arising from the breakdown of vegetation). During chlorination, these organics react with chlorine to form trihalomethanes, which are potentially carcinogenic and, therefore, undesirable in potable water. Thus, the removal of natural organics or trihalomethane precursors prior to chlorination would benefit water consumers. Synthetic organic compounds are the other major group of compounds requiring removal. Synthetic organics are compounds manufactured by man and some of these compounds (e.g. the polychlorinated dibenzo-p-dioxins) are highly toxic to life forms. Because they are usually present in low concentration, they are often referred to as micropollutants.

Potable water treatment plants in Canada typically chlorinate their water for disinfection, and coagulate, settle and/or filter their water for turbidity removal. Separate treatment for synthetic organic removal (eg. by activated carbon adsorption) is normally not utilized in Canada. Results of some previous studies indicated that coagulation may have the potential to remove matural organics and synthetic organic chemicals in a cost effective manner (Young and Singer, 1979, Fung 1978). Furthermore, recent advances in coagulation chemistry made available a series of new type of coagulants containing prepolymerized gels believed to be particularly promising in organic removal (Dassonville, 1977/Fiessinger and Bersillon, 1977). This study was undertaken to try to determine the potential of coagulation for cost effective organics removal. In particular, this study aimed to evaluate the overall organic removal performance of conventional and new coagulants and coagulant aids, and thereby, try to find ways to improve organic removal without the use of an additional treatment step.

COAGULANTS

The most widely used coagulants are based on aluminum or iron salts. New synthetic organic products such as ionic polyelectrolytes are also finding some use.

The most commonly used coagulant in drinking water treatment is alum. Alum is the aluminum sulphate salt, $Al_2(SO_4)_3.18H_2O$. When added to natural water this salt hydrolyzes to the trihydroxide $l(OH)_3$ which precipitates.

In recent years, several alternative aluminum-based coagulants have been developed in Japan and France. These products have similar principles underlying their design. In France, The Societe Lyonaise des Eaux et de l'Eclairage developed Poly Basic Aluminum Chloride (PBAC) (Fiessinger and Bersillon, 1977). This coagulant is not stable with time, and must be manufactured at the site of its use. The composition of this product is not predetermined, and can be "tailored" according to need, by varying the stoichiometric ratio OH/Al. This value determines the relative amount of different polymeric and monomeric Al-OH species as shown in Figure 1. As the OH/Al ratio increases the size of the Al-OH species increases. Thus for PBAC 1.8, the predominant species

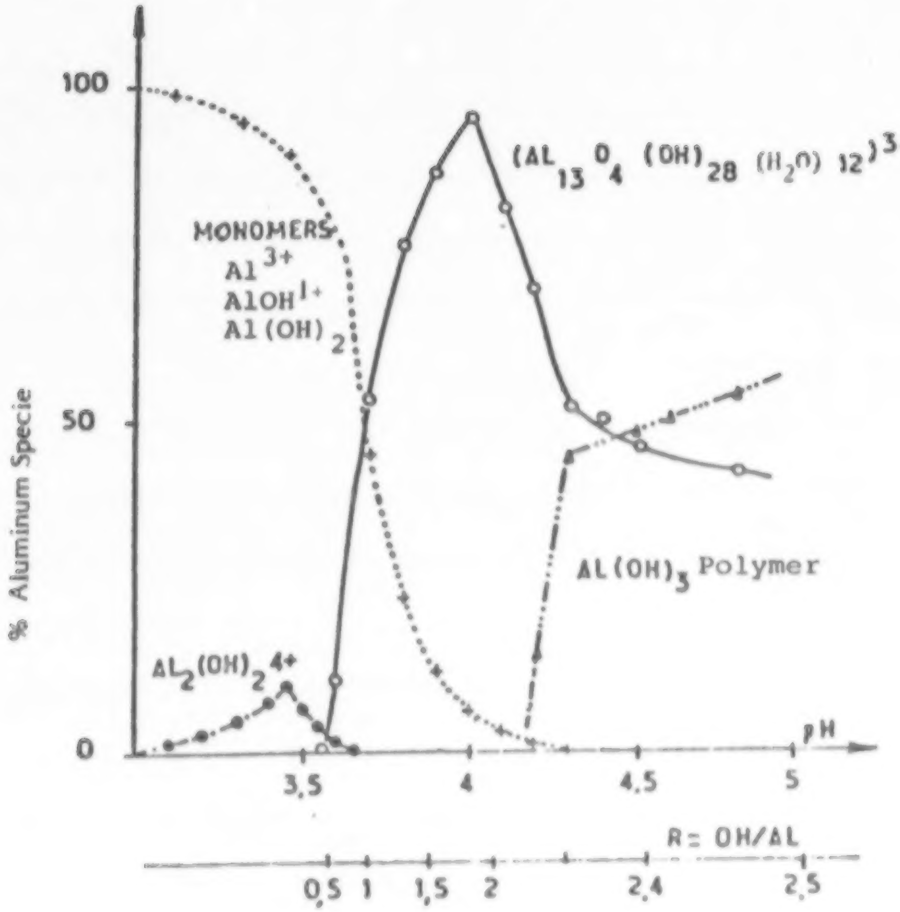


Figure 1 Aluminum speciation as a function of the OH/Al ratio. (Bottero, 1980)

is $(Al_{13}O_4(OH)_{28}(H_2O)_{12})^{3+}$ and for PBAC 2.2 , the aluminum atoms are approximately 50% in the $(Al_{13}O_4(OH)_{28}(H_2O)_{12}^{3+}$ form and 50% in the $Al(OH)_3$polymer form. Finally, in the case of PBAC 2.5, the $Al(OH)_3$ polymer becomes the dominant aluminum species.

In Japan, Taki Fertilizer developed Poly Aluminum Chloride Sulfate (PACS) (Taki, 1969) which has the overall composition of $Al(OH)_{1.8}(Cl,SO_4,PO_4)_{1.2}$. The active species is an aluminum hydroxy polymer, stabilized by the presence of the polyanions SO_4 or PO_4. This polyanion enhances the polymerization of the hydroxy polymers without precipitating them. The resulting active species has been found to precipitate quickly with an excess of sulfate which, according to Bersillon et al. (1980), indicates the large size of the polymer. PACS is currently widely used in Japan for the treatment of drinking water.

Ferric chloride is used extensively in wastewater treatment and can be used as an alternative coagulant in drinking water treatment (Carnduff, 1981).

All these coagulants, including the most conventional, i.e., alum, are mineral, and their common property is to hydrolyze to a final product (a trihydroxide) which precipitates, thereby entrapping the particulate material contained in the water.

In addition, organic coagulants have been developed as well. They are polyacrylamides or polyamines, and they are characterized by their charge (anionic, cationic, nonionic), their molecular weight, and their degree of dissociation (Benedek et al., 1977).

METHODOLOGY

In this study organic removals were evaluated with the aid of jar tests (bench-scale coagulation tests) on three typical Ontario water sources, namely:

(i) Lake Ontario water at Hamilton
 - typical of Great Lakes water

(ii) Groundhog River water at Fauquier
 - typical of water from the Canadian Shield

(iii) Grand River water at Brantford
 - typical of water from an industrially polluted sedimentary basin.

Table 1 shows the averaged values of selected physical, chemical and organic parameters for the three raw waters used in this study.

The jar tests were performed with a bench scale flocculator, as described by Benedek (1976). This method allows for the determination of floc settlability in addition to the determination of the physical, chemical and organic characteristics of the treated water. The tests were conducted according to the flow chart shown in Figure 2.

In addition to jar tests, larger-scale batch coagulations, and small-scale continuous filtrability evaluations were performed at a limited number of treatment conditions on Brantford water.

The natural organic concentration was measured as Dissolved Organic Carbon (DOC) on a Dohrman DC54 TOC Analyzer (Dohrman Co., Santa Clara, Calif.) and as Ultraviolet Absorbance at 254nm (A_{254nm}) in quartz cells using an empty

TABLE 1

AVERAGED VALUES OF THE RAW WATER CHARACTERISTICS

	Hamilton	Brantford	Fauquier
pH	7.96	8.48	7.98
Alkalinity mg/L	87.5	250	70.0
Turbidity NTU	0.96	3.30	1.71
UV Abs. (254 nm)	0.300*	0.258**	0.644**
DOC mg/L	2.34	6.44	14.9
TTHMFP ug/L $CHCl_3$	74.4	351	765***

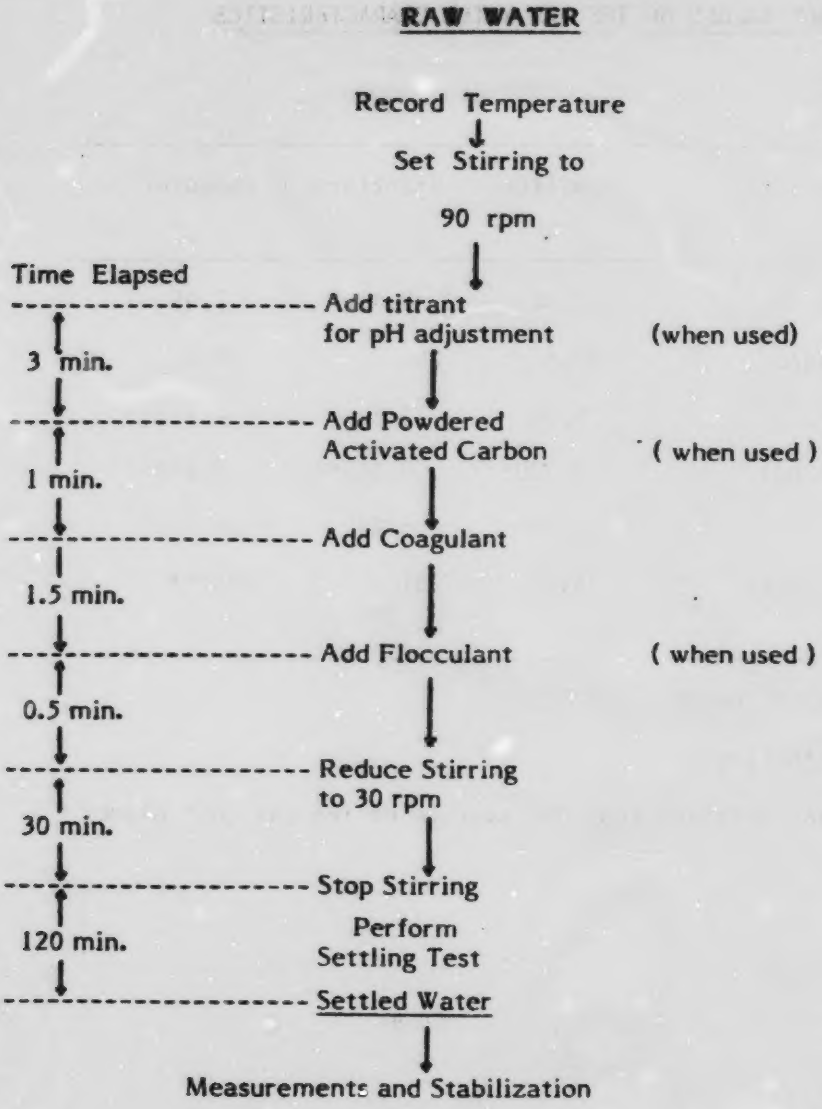
* 10 cm path length

** 1 cm path length

*** value was obtained from the average of the jar test blanks

Figure 2

JAR TEST FLOW CHART


RAW WATER
Record Temperature
Set Stirring to
90 rpm
Time Elapsed
Add titrant
for pH adjustment
(when used)
3 min.
Add Powdered
Activated Carbon
(when used)
1 min.
Add Coagulant
1.5 min.
Add Flocculant
(when used)
0.5 min.
Reduce Stirring
to 30 rpm
30 min.
Stop Stirring
120 min.
Perform
Settling Test
Settled Water
Measurements and Stabilization

cell as the reference as described by Peel (1980), using a Bausch and Lomb Spectronic 21 Spectraphotometer (Bausch and Lomb Co., Baton, N.Y.).

The trihalomethane precursor concentration was evaluated in terms of the Total Trihalomethane Formation Potential (TTHMFP) as described by Stevens et al 1980. Figure 3 shows the relationship between the TTHMFP and the THM's formed during conventional treatment with raw water chlorination and the effect of water treat[illegible]n TTHMFP. The THM analysis was according to the method used by Henderson et al.,(1976) and the gas chromatograph used was a 5880 Hewlett Packard (Hewlett Packard Co., Avondale, Pennsylvania) equipped with an automatic sample injector and electron capture detector.

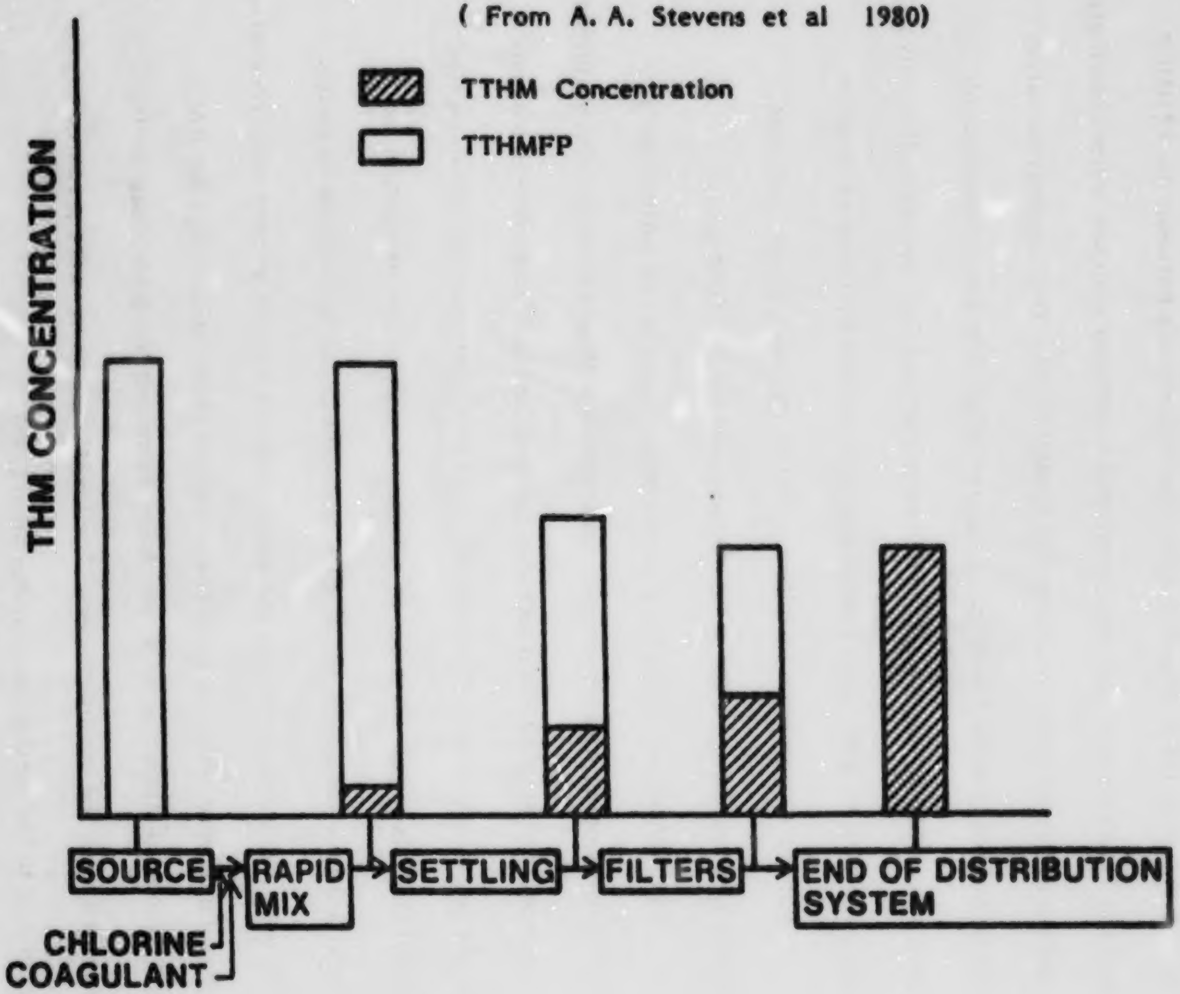
For the examination of spiked synthetic organics the analysis was performed according to the same method used for the THM analysis.

Examination of synthetic organics present in the raw water supply and corresponding treated samples commenced with an initial gas chromatograph/ mass spectrometer (GC/MS) full scan analysis for qualitative determination of the organics present. This was followed by GC/MS multiple ion detection (MID) analysis for quantitation of selected organics. The extraction for these analysis was conducted by EPA Method 625 for Extractable Organics. The data was blank corrected and normalized for d_{10} anthracene spike recovery. Coagulants were prepared from reagent grade chemicals, except for PACS and Cyanamid Magnifloc 572C which were commercial grade coagulants obtained from the manufacturers. The PBAC coagulants were prepared according to the method used by Bersillon (1977).

FIGURE 3

TRIHALOMETHANES FORMED DURING CONVENTIONAL TREATMENT WITH RAW WATER CHLORINATION

(From A. A. Stevens et al 1980)



RESULTS & DISCUSSION

ORGANIC PARAMETER CORRELATIONS

Natural organics were measured in this study by Dissolved Organic Carbon (DOC) as well as Ultraviolet Absorbance at 254 nm ($A_{254\ nm}$).

Figures 4 to 6 show the measured DOC as a function of the absorbance at 254 nm, for each water. The path length had to be adjusted as a function of the organic content of the samples. For Hamilton water, a 10 cm path length was adopted, throughout the experiments, whereas the samples from the experiments on Brantford and Fauquier water were measured at a 1 cm path length.

The results show a strong correlation between the UV absorbance at 254 nm and the DOC for Brantford and Fauquier water. This correlation is weaker in the case of Hamilton water.

For each water sampled a UV-DOC correlation curve was constructed and the UV absorbance data was transformed into DOC (mg/L) for further discussion.

When natural organics are chlorinated trihalomethanes are formed. Thus the Total Trihalomethane Formation Potential (TTHMFP) is another measure of the fraction of natural organics that can act as precursors in the trihalomethane formation reaction. A strong correlation was found to exist between the DOC and the TTHMFP for both Brantford and Fauquier water, as shown in Figure 7. When both sets of data are plotted on the same graph, they seem to belong to the same straight line. The interpretation follows:

i) The reactivities of the organic content of Brantford

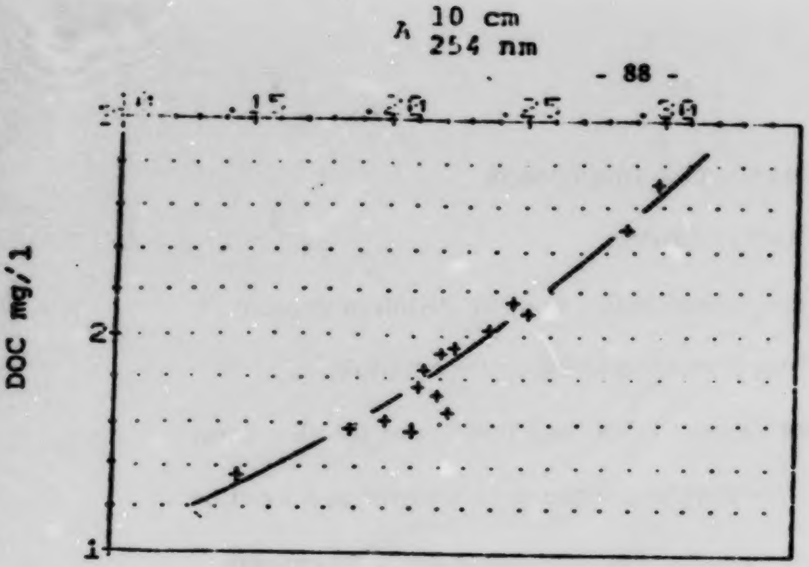


Figure 4
Correlation between UV Absorbance and DOC for Hamilton Water.

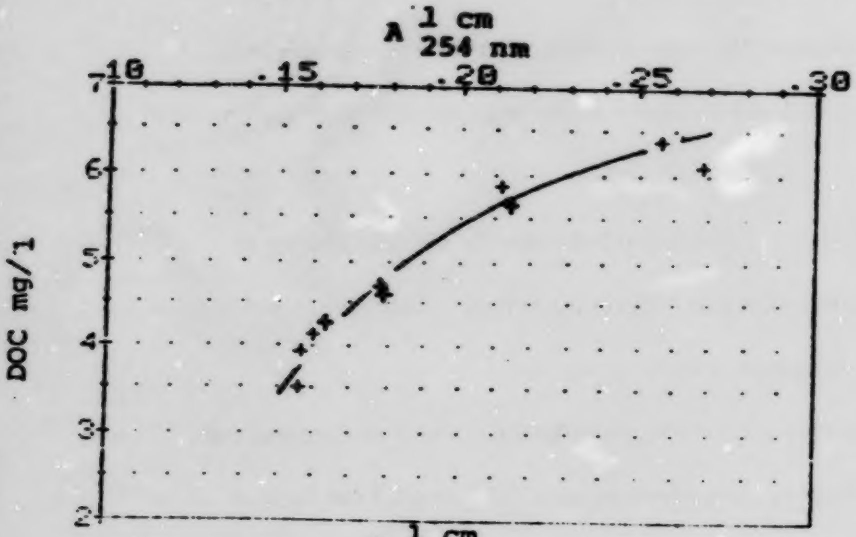


Figure 5
Correlation between UV Absorbance and DOC for Brantford water.

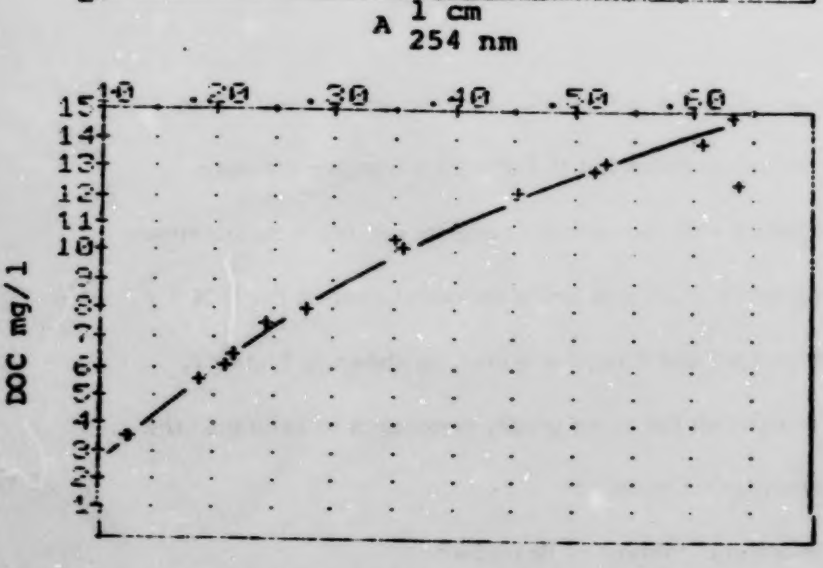
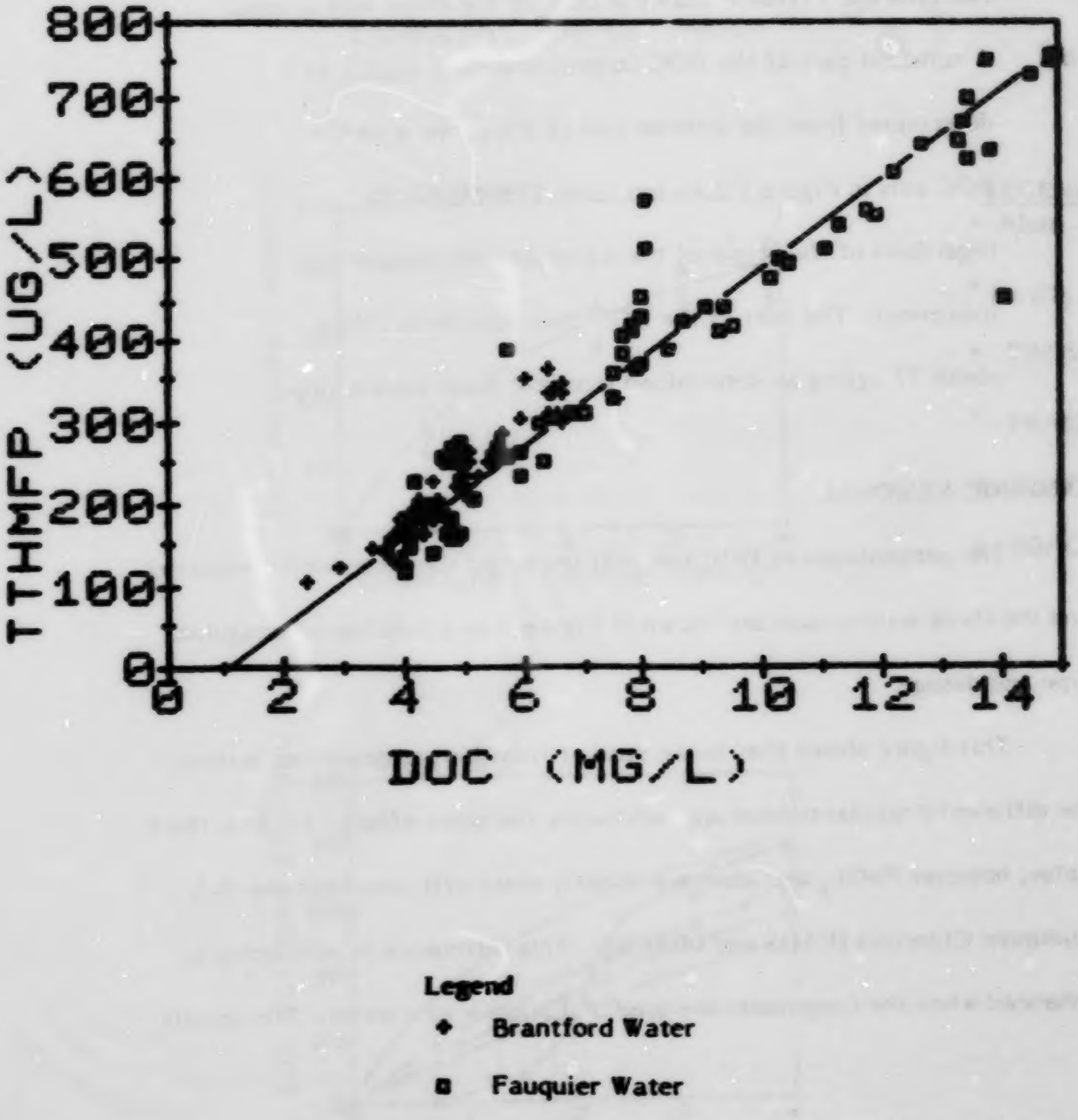


Figure 6
Correlation between UV Absorbance and DOC for Fauquier water.

Figure 7 TTHMFP as a Function of DOC for Brantford and Fauquier Waters



and Fauquier water with regard to chlorine are equal within the experimental conditions

ii) The interaction of the different coagulants with the organic material is not selective, as no coagulant better removes the TTHMFP than the DOC or the other way around.

iii) A constant part of the DOC (approximately 1 mg/L), as determined from the intersection of the curve with the DOC axis in Figure 7 does not form TTHM's, regardless of the origin of the water and the coagulation treatment. The part of the DOC that does form THMs yields 57 ug/mg as determined from the least square slope.

ORGANIC REMOVAL

The percentages of DOC removal for six of the coagulants evaluated and the three waters used are shown in Figure 8 as a function of coagulant type and dosage.

This figure shows that in the case of Hamilton or Brantford waters, the different coagulants have approximately the same effect. On Brantford water, however $FeCl_3$ and alum are slightly more efficient than the Poly-aluminum Chlorides (PACS and PBAC's). This difference in efficiency is enhanced when the coagulants are used on Fauquier raw water. The results

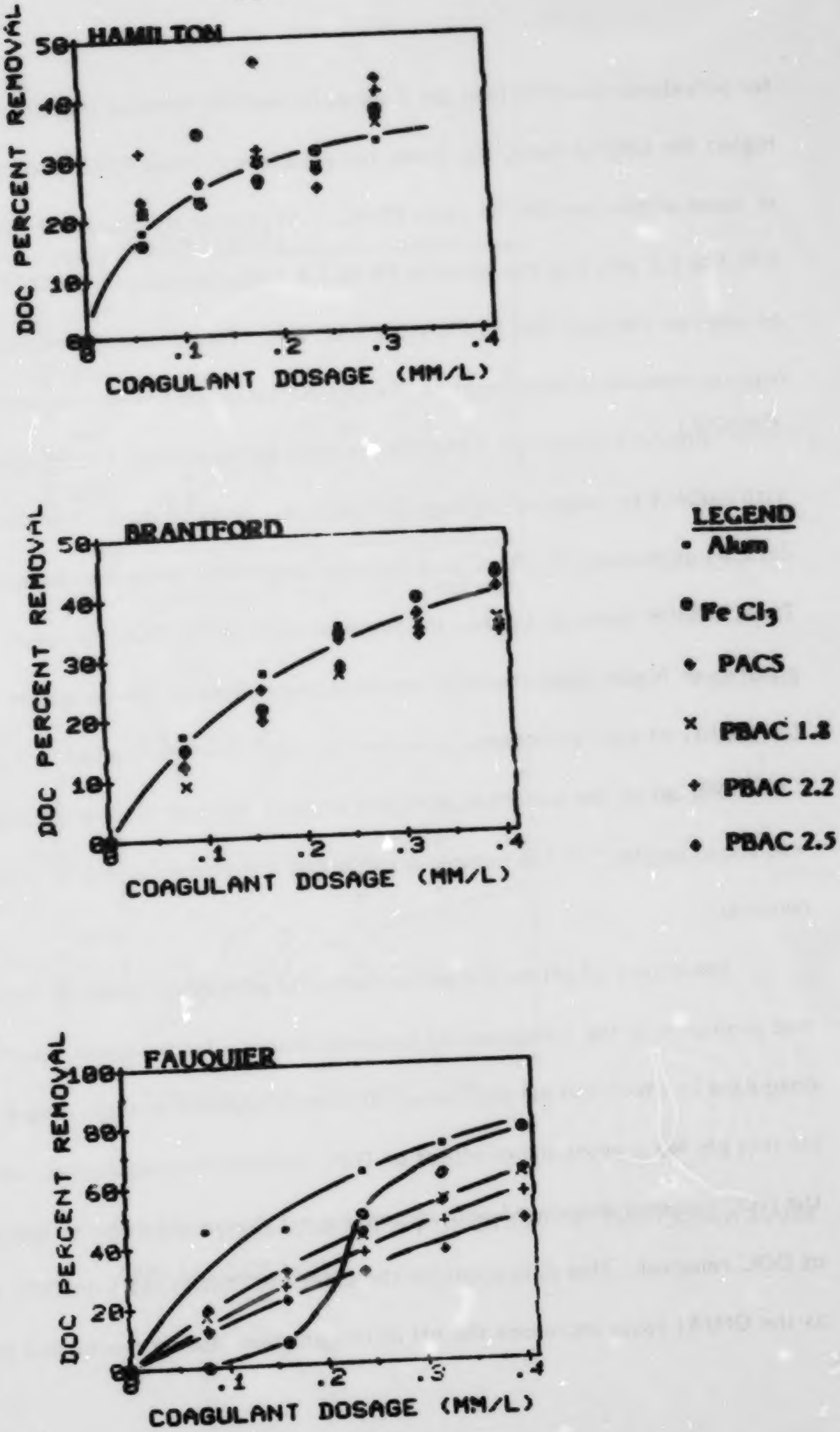


FIGURE 8 DOC REMOVAL AS A FUNCTION OF COAGULANT TYPE AND DOSAGE

for polyaluminum chlorides are then split, and the trend is that for PBAC the higher the OH/Al ratio, the lower the efficiency, while PACS shows removals similar to those of the low OH/Al ratio PBAC. As previously discussed the OH/Al ratio of PACS is 1.8 which is the same as PBAC1.8 while the size of the PACS polymer is as large as the high OH/Al PBAC, PBAC 2.5. Thus this data indicates that natural organic removal is dependent on the OH/Al ratio but not on the polymer size.

Figure 9 shows the TTHMFP removal for Brantford and Fauquier water with respect to coagulant dosage for the coagulants studied. The Hamilton water data is not included in this figure because analytical problems obscured overall trends. The TTHMFP removal follows the same pattern as the DOC removal, and it is equal to or higher than the DOC removal, regardless of the coagulant used or the quality of the raw water.

For all of the waters studied the organic polymer Cyanamid Magnifloc 572C was found ineffective for natural organic and trihalomethane precursor removal.

The effect of pH on the performance of alum, PACS, PBAC 1.8 and $FeCl_3$ was evaluated in the treatment of Fauquier water. Jar tests were performed for each coagulant in which the pH was varied at fixed coagulant dosage. Figure 10 shows that jar test pH has a pronounced effect on DOC removal in coagulation; above pH 6 the DOC removal decreased with increasing pH, below pH 6 there is an apparent plateau of DOC removal. This data explains the effect of OH/Al ratio on DOC removal since as the OH/Al ratio increases the pH of coagulation also increases and thus the DOC

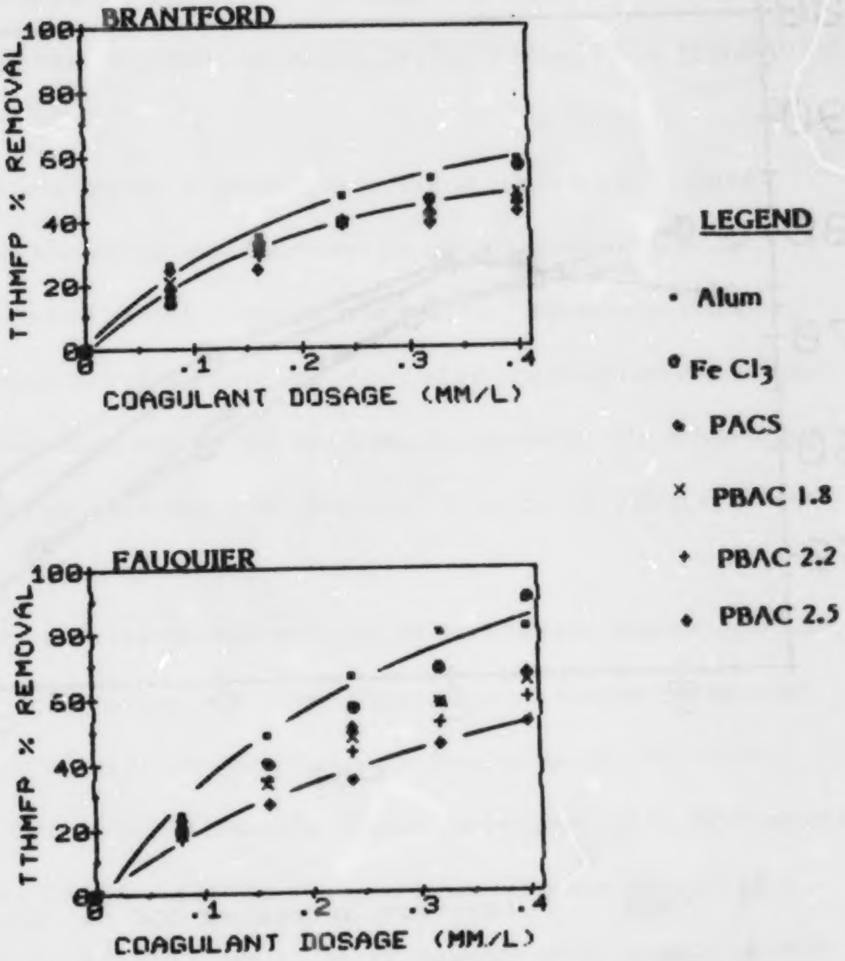
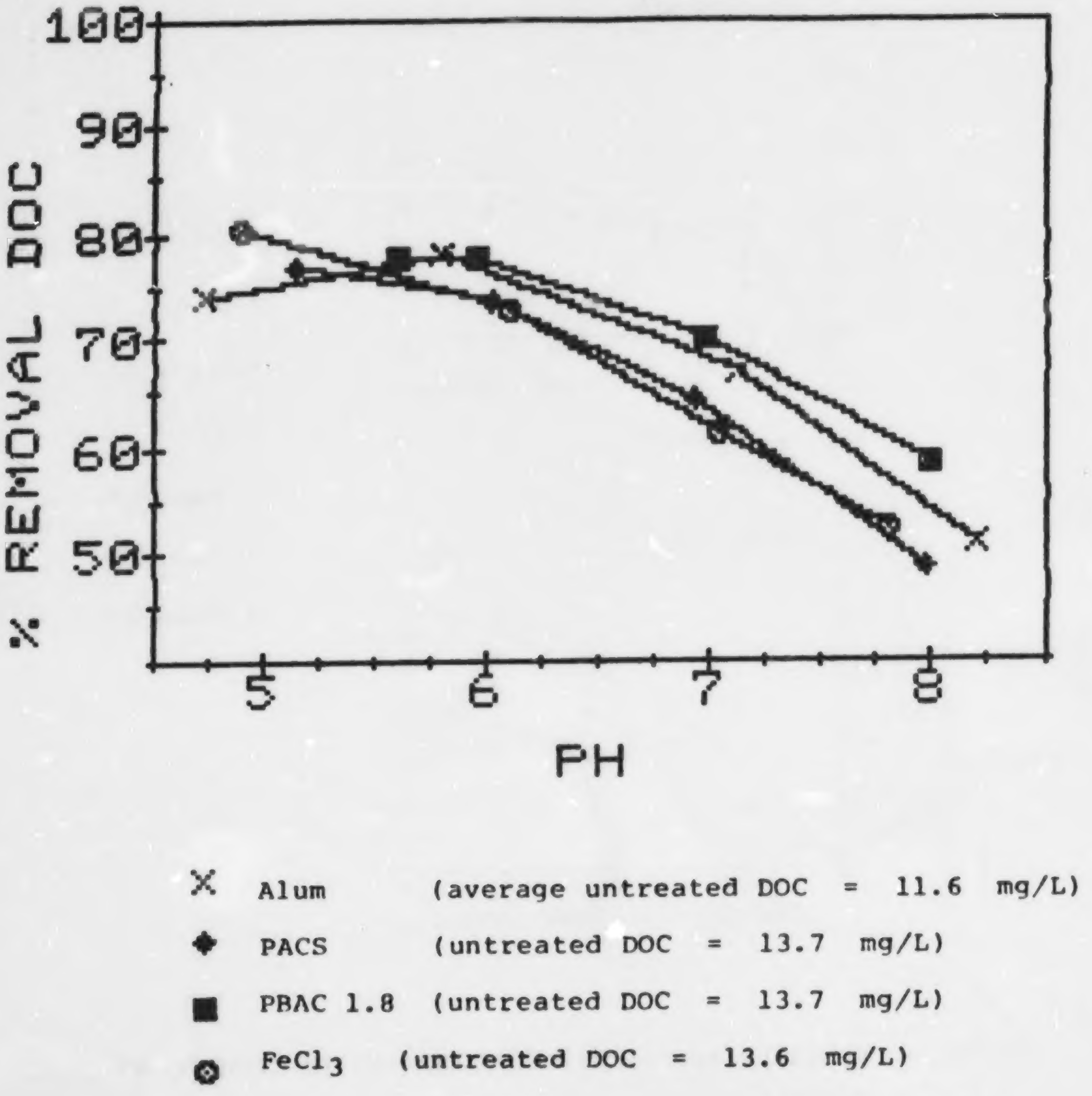


FIGURE 9 TTHMFP REMOVAL AS A FUNCTION OF THE COAGULANT AND ITS DOSAGE.

Figure 10 Percent Removal of DOC as a Function of pH for Fauquier water



removal per mole of aluminum decreases.

Figure 11 summarizes the percent removal of TTHMFP at the working optimum dosage at various pH's for the four coagulants examined. The results shows that for all four coagulants decreasing pH results in increasing TTHMFP removal.

The significant effect of pH on natural organic removal and TTHMFP removal suggests that coagulation treatment for organic removal should be optimized with respect to both coagulant dose and pH. Therefore in this study an in-depth examination of the combined effect of pH and coagulant dosage was performed for two water supplies and two types of coagulants. The effect of pH and PBAC 1.8 dosage was examined on Brantford water and the effect of pH and alum dosage was examined on Fauquier water.

For both waters examined an optimum pH was observed where a significant improvement in DOC removal was noted. Figure 12 shows the percent removal of DOC as a function of pH and alum dosage for Fauquier water. The figure shows that for Fauquier water treated with alum the optimum pH for DOC removal is approximately 5.7 and that at this pH the optimum coagulant dosage, (where there is a 5% reduction in DOC per 0.1 mM of coagulant added) is approximately 0.20 mM/L. A very similar pattern of DOC removal was observed for Brantford water treated with PBAC 1.8. The optimum pH for this water and coagulant was 6.5 and the optimum coagulant dosage was approximately 0.32 mM/L. The

Figure 11 Percent Removal of TTHMFP as a Function of pH for Fauquier water

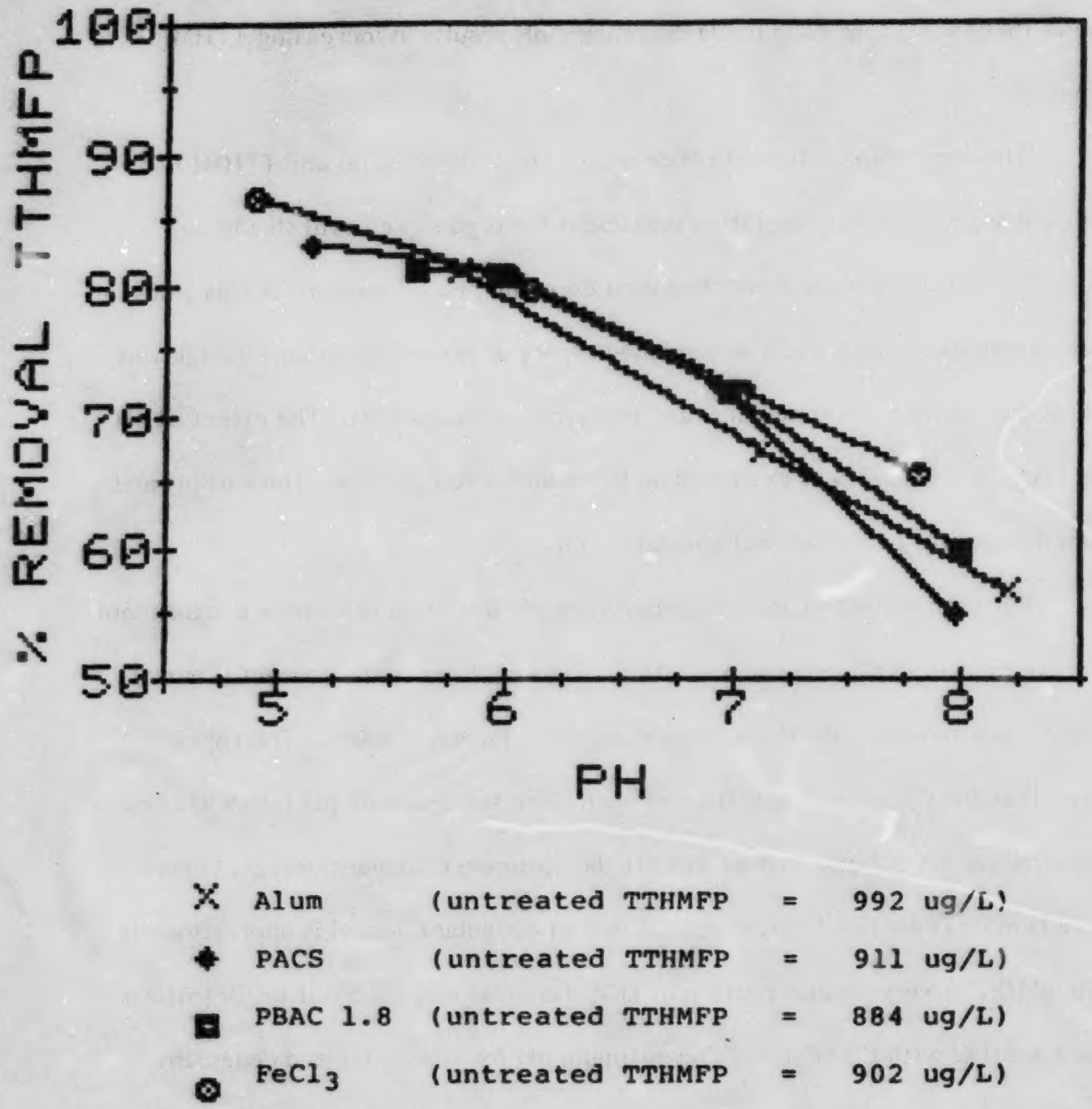
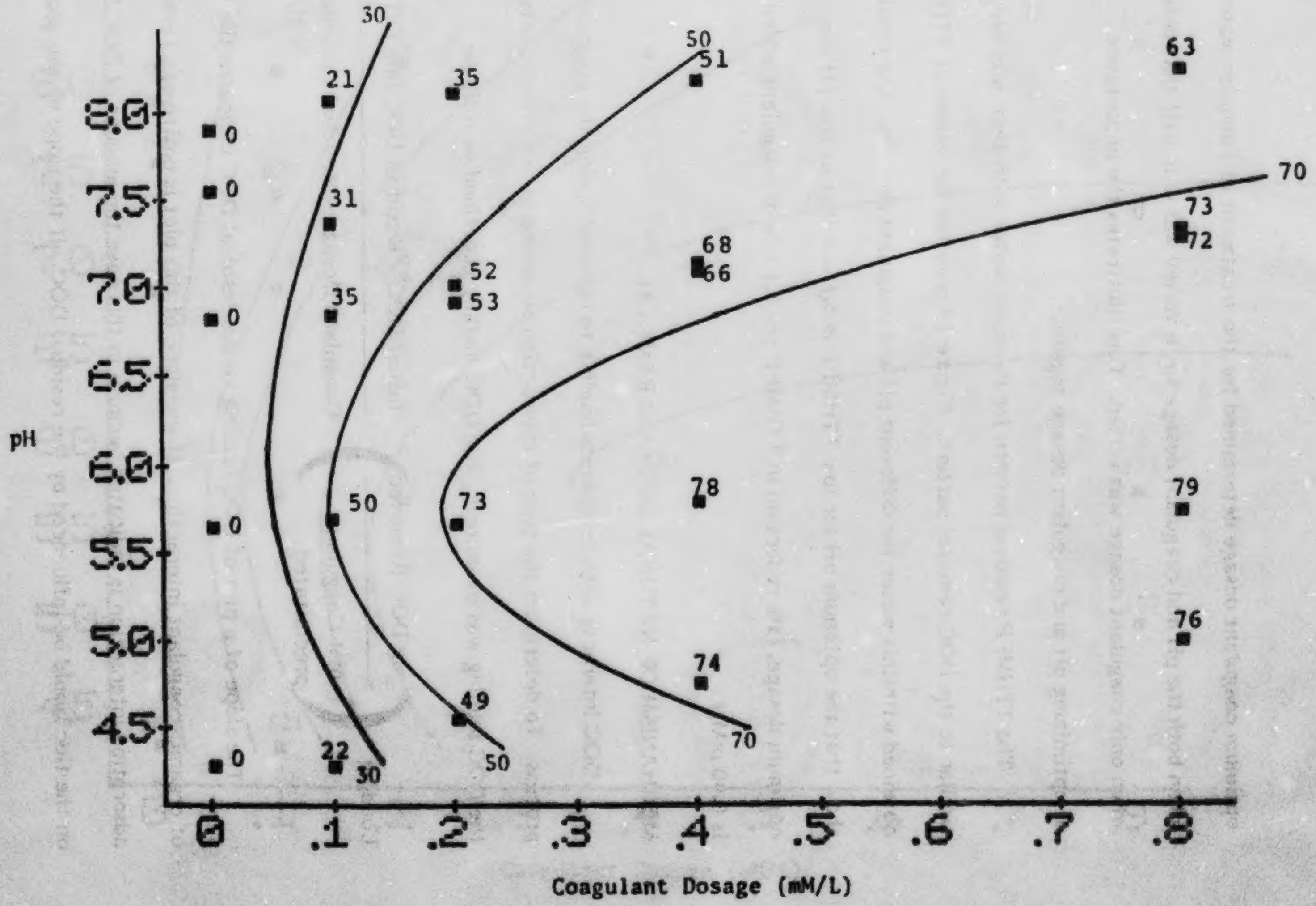


Figure 12 The percent removal of DOC as a function of pH and Alum dosage for Fauquier water (Average untreated DOC = 11.6 mg/L)



optimum coagulant dosage determined for the treatment of Fauquier water when both the pH and coagulant dosage were varied was only half that determined when only coagulant dosage was varied. This illustrates the importance of optimizing pH and coagulant dosage together.

The TTHMFP removal pattern for Fauquier water with alum was very similar to the DOC removal pattern. Figure 13 presents the values of TTHMFP obtained with this water for different pH and coagulant dosages. The results show that the optimum pH for low TTHMFP is 5.7 and that at this pH the optimum dosage, (5% reduction in TTHMFP per 0.1 mM of coagulant added) is 0.40 mM/L.

MECHANISM OF NATURAL ORGANIC REMOVAL

DOC interacts with coagulants leading to removal during the coagulation process. To determine the type of interaction occurring in our experiments, the DOC loading was examined. The DOC loading is defined as follows:

$$\text{Loading}\left(\frac{\text{mg DOC Removed}}{\text{mM Coagulant precipitated}}\right) = \frac{\text{Initial DOC - Residual DOC (mg C/L)}}{\text{Coagulant Dosage - Residual Coagulant (mM/L)}}$$

The slope of a plot of DOC loading versus residual DOC indicates the type of organic-coagulant interaction. If the slope of this plot is positive, a reversible adsorption interaction is indicated because in this case the amount of DOC "loaded" on the floc would be influenced by the residual DOC. If the slope of this plot is

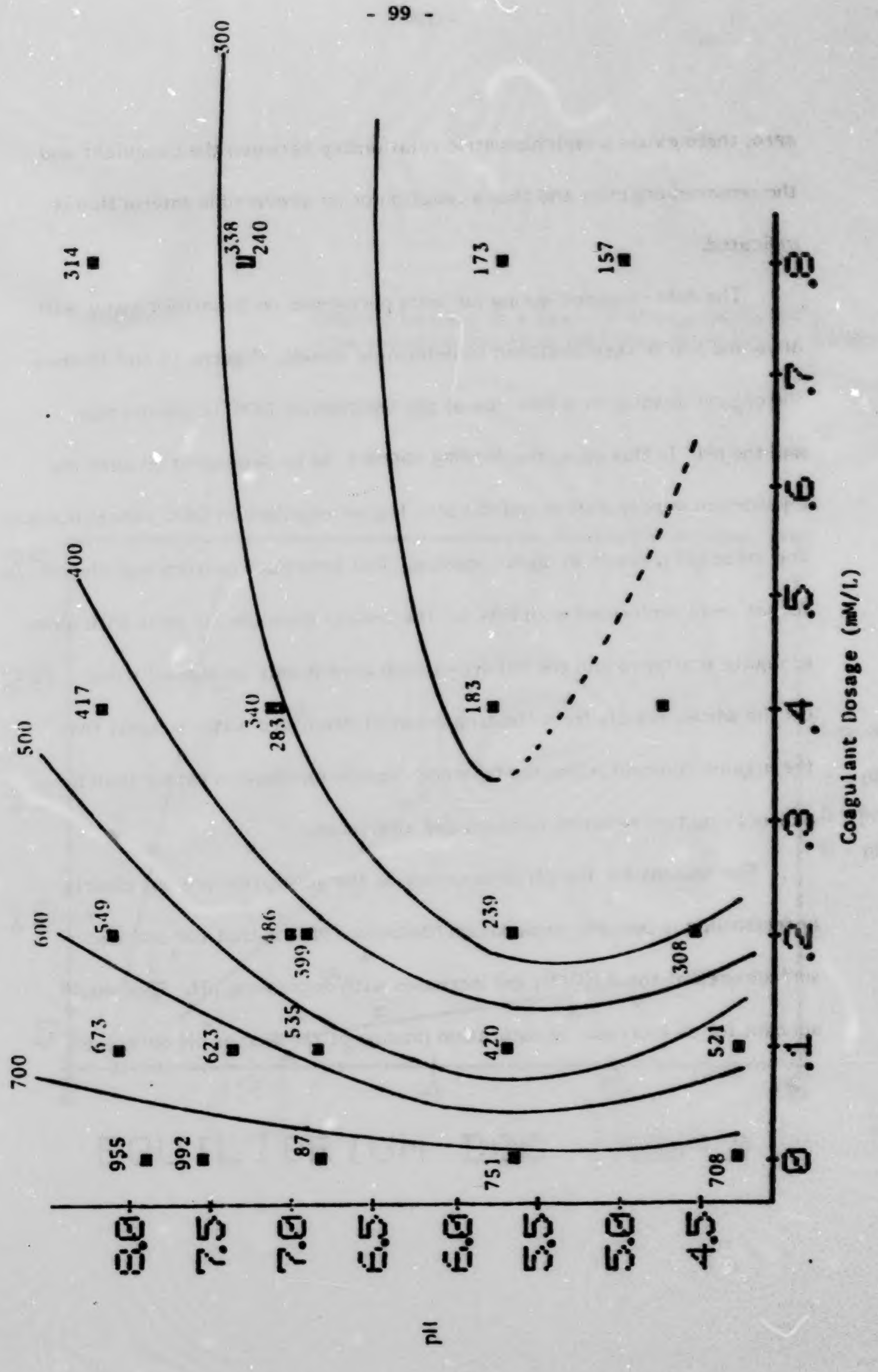


FIGURE - 13 TTHMFP (ug/L) as a function of pH and alum dosage for Fauquier water zero, there exists a stoichiometric relationship between the coagulant and the removed organics and thus a reaction or an irreversible interaction is indicated.

The data colected during jar tests performed on Brantford water with alum and PACS were analyzed to determine trends. Figures 14 and 15 show the organic loading as a function of the equilibrium DOC concentration and the pH. In this case, the loading appears to be dependent on both the equilibrium concentration and the pH. Higher equilibrium DOC concentrations and lower pH's, result in higher loading. This behaviour pattern was clearer for jar tests performed with PACS. The results from the jar tests with alum are quite scattered and the behaviour indicated is only an overall tread. On the whole, results from the treatment of Brantford water suggest that the organic removal is limited by an adsorption mechanism rather than by a direct reaction between fulvates and aluminum.

The reasons for the pH dependency of the adsorption are not clearly understood, one possible explanation (Bottero, 1981) is that the specific surface area of the $Al(OH)_3$ gel increases with decreasing pH. This would account for an increase in saturation loading of the floc as pH decreases.

Figure 14 **Organic Loading as a function of Equilibrium DOC concentration for Alum treatment of Brantford Water**

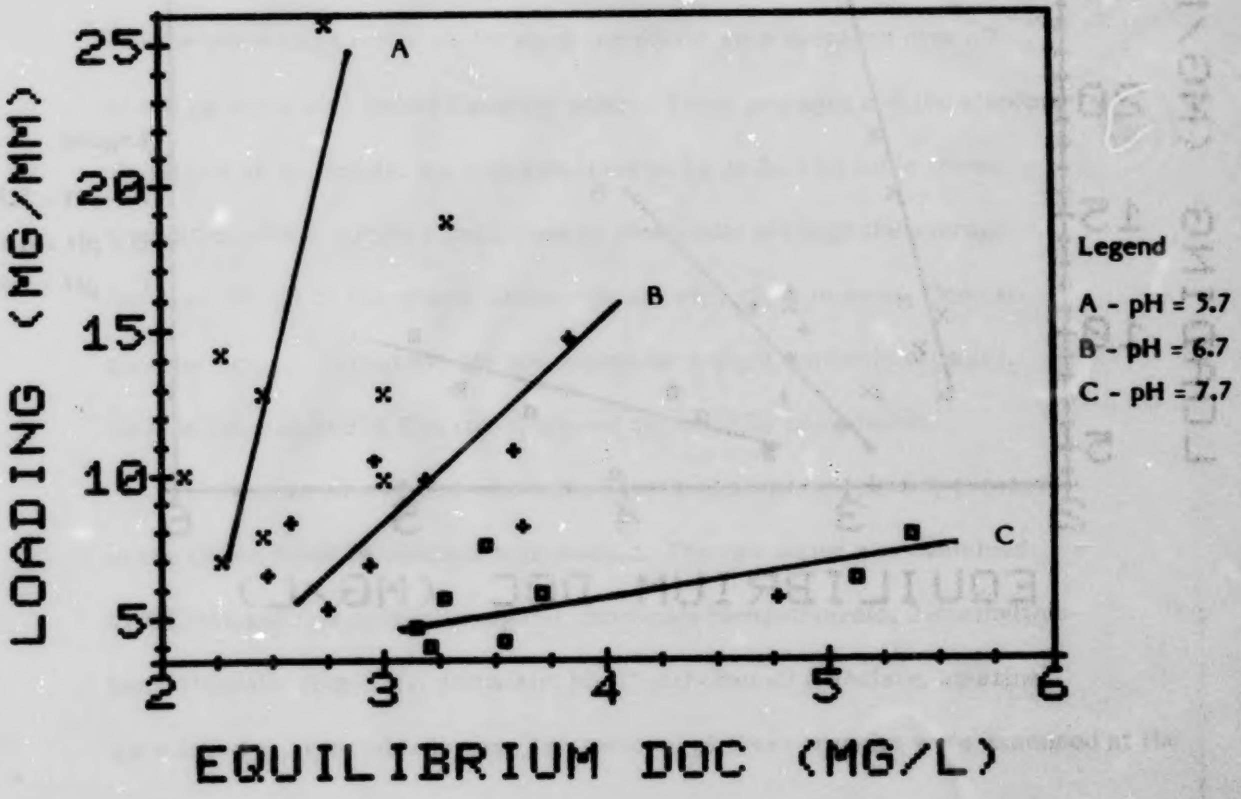
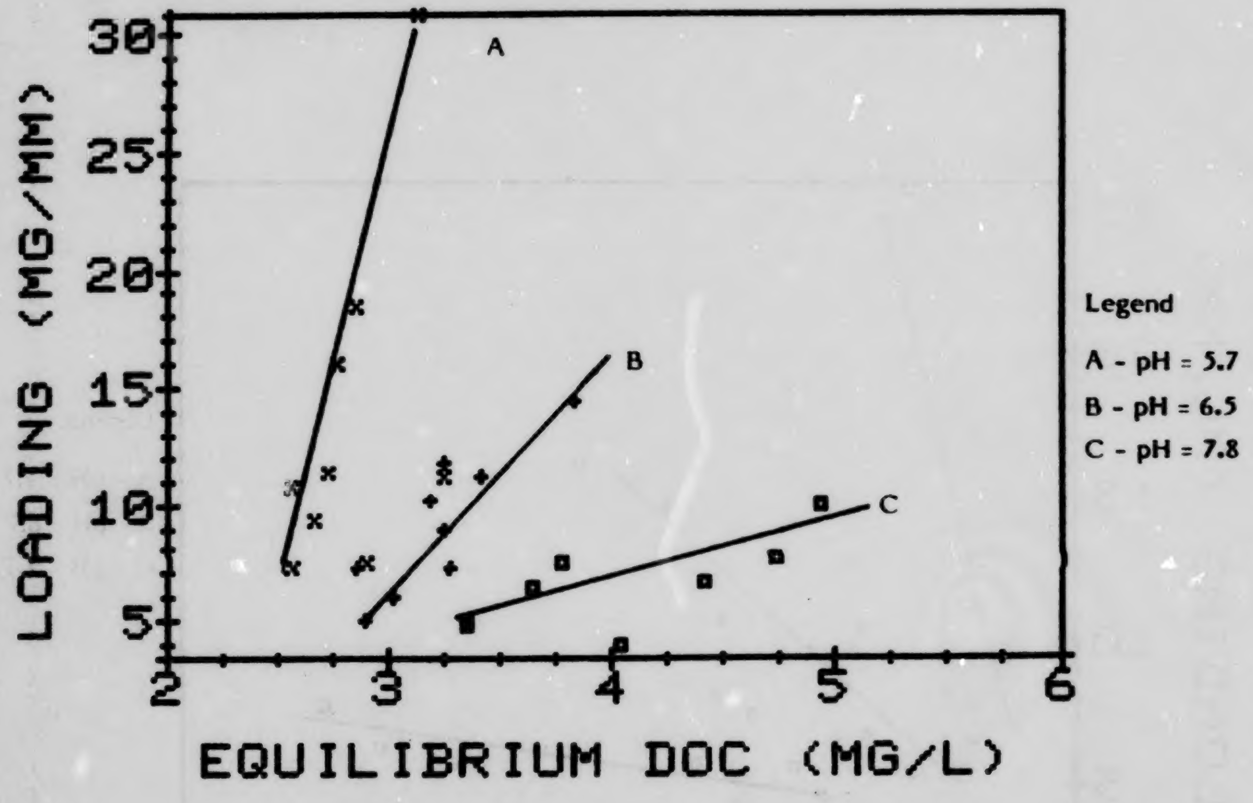


Figure 15 **Organic Loading as a function of Equilibrium DOC concentration for PACS treatment of Brantford Water**



SYNTHETIC ORGANIC REMOVAL

Two approaches were used to investigate removal of synthetic organics. In the first case selected synthetic organic chemicals (Table 2) were spiked into Fauquier water and their removals evaluated under 28 different pH/alum dosage combinations. Examination of the removal data showed that the percentage removals of the recoverable organics were in general between ±15% and appeared to be random with respect to pH and alum dosage. Thus it was assumed that pH and alum dosage did not affect the observed removals and the percentage removals for each compound were averaged over all of the jar tests with spiked Fauquier water. These averages and the standard deviations of the results are also tabulated in Table 2. This table shows that although the standard deviations of the results are high the average removals for all of the spiked compounds are very close to zero. One can, therefore conclude that soluble low molecular weight synthetic organics, such as those spiked in this study, are not removed by coagulation.

In the second case the removals of synthetic organics already present in the Grand River (Brantford) was studied. The raw water was examined by GC/MS and five synthetic organic chemicals [benzothiazole, 2-methylthiobenzothiazole, di-n-butyl phthalate, bis-(2-ethylhexyl) phthalate, atrazine] were found at detectable levels. The removal of these organics were examined at the

TABLE 2

AVERAGED PERCENTAGE REMOVALS OF SPIKED ORGANICS IN FAUQUIER WATER

Compound	Average Percentage Removal ± Standard Deviation	Number of Points	Analytical Reproducibility
Chloroform	2.2 ± 12.1	28	± 10%
1,1,2-Trichloroethane	-1.6 ± 14.6	28	± 10%
Toluene	1.8 ± 11.8	22	± 10%
Naphthalene	1.6 ± 9.8	22	± 10%
2,4-Dichlorophenol	-3.6 ± 27.0	27	± 10%

optimum treatment conditions with alum and with PACS. In this case variability in chemical analysis (due to limitations in current analytical techniques), and matrix changes (caused by coagulation affecting analytical recoveries) may have obscured small removals. The analytical variability ranged from ± 21% for atrazine to ± 74% for bis-(2-ethylhexyl) phthalate. Removals of these synthetic organics at levels greater than the analytical variability were not observed.

OPTIMUM ORGANIC REMOVAL

So far as natural organic and TTHMFP removal is concerned this study has shown that there is no true optimum coagulant dosage and pH, since organic removal continuously increases with increasing dosage and increases with decreasing pH. Potentially, coagulant dosage may be adjusted to reach a predetermined residual organic concentration. Alternatively, the dosage for which an additional increment of coagulant would lead to marginal improvement of the organic residual can be defined as the optimum (for this study this was defined as the dosage at which 0.1 mM/L of additional coagulant resulted in less than 5% reduction of residual organics).

Furthermore, there are other operational factors which must be taken into account in the coagulation step, such as the settling velocity of the floc, the residual turbidity in the clarifier overflow and the residual coagulant concentration.

These parameters were evaluated in the jar tests together with the organic and TTHMFP removals. They were taken into account indirectly by setting maximum or minumum acceptable values based on regulatory guidelines or limitations based on water treatment plant operations. The working criteria used are summarized in Table 3. In general it was found that in the pH and coagulant dosage range for which organic removal was optimized all coagulants removed the turbidity satisfactorily. However, the flocs produced by the prepolymerized coagulants (PACS and PBAC) were easier to remove by settling and also by filtration.

Results consistently indicate that careful attention should be given to the operating pH during coagulation as it both determines the amount of residual aluminum and significantly affects the extent of organic and TTHMFP removal. The coagulation step should be performed at a pH within the minimum solubility band of the aluminum ion, to avoid problems in the distribution network, due to delayed precipitation.

Performance evaluation of four of the coagulants, (alum, $FeCl_3$, PACS and PBAC 1.8) on Fauquier water sampled in one season, resulted in selection of alum and PACS for more intensive study on Grand River water. Both of these coagulants, when used on Fauquier water at pH 6.0 and a dosage of 0.4 mM/L, achieved good natural organic removal (as measured by Dissolved Organic Carbon) and trihalomethane precursor removal (as measured by TTHMFP); residual turbidity, settling velocity and residual aluminum were all acceptable. Furthermore, both coagulants removed approximately 25% more of the natural organics and TTHMFP

TABLE 3

Working Criteria Used to Evaluate Coagulant Performance

	Maximum	Minimum
pH	8.5	6.5
Residual Aluminum (mg/L)	0.10	--
Residual Iron (mg/L)	0.30	--
Settling Velocity m/h	--	0.2
Residual Turbidity (NTU)	5	--

than is being currently removed at the Fauquier water treatment plant. To obtain these improved results, coagulant dosage at the plant would have to be approximately doubled and the pH of the water adjusted before and after treatment.

Studies were conducted on Brantford water sampled in three different seasons. The optimum dosage of alum or PACS ranged between 0.24 mM/L and 0.30 mM/L depending on the season, and the optimum pH ranged between 6.5 and 6.9. Under these conditions coagulation resulted in up to 47% more removal of the DOC and up to 78% more of the TTHMFP than the coagulation treatment currently being used at the Brantford water treatment plant; residual turbidity, settling velocity and residual aluminum were all acceptable. With respect to organic removal, PACS performed marginally better than alum. However, the settling velocities and residual turbidities in water treated with PACS were improved relative to the values obtained with alum treatment. To obtain these improved organic removals, a coagulant dosage 5 to 6 times greater than the alum dosage currently applied at Brantford would have to be used and the pH of the water would have to be adjusted before and after coagulation.

CONCLUSIONS

i) Coagulation can be a very efficient method for the removal of the natural organic material and for the removal of trihalomethane precursors.

ii) Coagulation treatment conditions can be optimized, with respect to both pH and coagulant dosage, to improve natural organic and trihalomethane precursor removal.

iii) Coagulation was found to be ineffective for the removal of soluble synthetic organic compounds spiked into a raw water. In the case of synthetic organics already present in a raw water, analytical limitations did not allow a clear indication of removal effectiveness (despite the used of surrogate standards to account for method variability). However, removals due to coagulation of these synthetic organics at levels greater than the analytical variability were not observed.

iv) The operating mechanism for natural organic removal during the coagulation/flocculation process appears to be adsorption.

v) The role of pH is extremely important in the definition of optimum treatment conditions for inorganic coagulants as it significantly affects the extent of organic removal, trihalomethane precursor removal, and residual coagulant aluminum or iron concentration. Adjustment in pH alone, regardless of coagulant dosage can significantly improve organic removal and trihalomethane precursor removal.

vi) The three alternative inorganic coagulants examined (PACS, PBACS and ferric chloride) have similar removal efficiencies at optimum treatment conditions to the conventional coagulant, alum, for removal of organics and THM precursors. However, PACS produced a floc with better settling characteristics and resulted in a lower residual turbidity.

vii) The organic polymeric coagulant (Cyanamid Magnifloc 572C) was not effective for removing organics or THM precursors.

viii) For improvement in the removal of natural organics and the reduction of TTHMFP in waters with high natural organic concentration, and low alkalinity, of which Fauquier water is typical, coagulation can be optimized without dramatically increasing the amount of chemicals required over those already used.

ix) For significant improvement in the removal of natural organics and the reduction of TTHMFP in waters with a moderate natural organic concentration, and high alkalinities, of which Brantford water is typical,significant increases are required in the amounts of coagulants used over the amounts currently used.

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ABSTRACT

A full-scale gravity flow UV system which consists of a series of modules forming two separate rectangular matrices of UV lamps was placed in each effluent channel of a conventional secondary sewage treatment plant.

Photoreactivation and dark repair experiments showed that 3h produced the maximum number of total and fecal coliforms whereas fecal Streptococci showed no increase.

Routine bacteriological sampling has shown that the geometric mean of the total and fecal coliform bacteria before and after photoreactivation can be maintained below the limits of 2,500 and 200 organisms per 100 ml respectively in between the matrices as well as after the two UV units. The chlorinated effluent was similar bacteriologically except for the resistant spore-forming bacteria, Clostridium perfringens, and bacteriophage of Escherichia coli C which were more sensitive to UV irradiation.

A fish toxicity evaluation conducted in the receiving stream showed that chlorinated effluent was toxic to rainbow trout whereas UV irradiated effluent was not.

A cost comparison has shown that UV disinfection is a cost effective alternative to other forms of wastewater disinfection such as chlorination, chlorinaton-dechlorination and ozonation.

The UV and chlorinated effluents have been tested for the following pathogens: Pseudomonas aeruginosa, Yersinia enterocolitica, Campylobacter jejuni and Salmonella sp.

ULTRAVIOLET DISINFECTION OF SECONDARY SEWAGE EFFLUENT

Garry Palmateer[1], G. Elliott Whitby[2], Walter G. Cook[1],
Jan Maarschalkerweerd[2], Doug Huber[1], Ken Flood[3]

1. Trojan Technologies, London
2. Ontario Ministry of the Environment, London
3. Ontario Ministry of the Environment, Toronto

The elimination of pathogenic microorganisms from wastewater before discharge to receiving waters which may be used for human or livestock purposes is of the utmost importance. In North America, disinfection of treated sewage is accomplished almost exclusively with chlorine. An increasing body of research[1,2,3,4] reveals that chlorine residuals in receiving waters are deleterious to the aquatic biota. Chlorination also produces chlorinated compounds that may be toxic to humans and animals[4]. An alternative to chlorination is ultraviolet (UV) disinfection[5].

To date, studies involving UV disinfection of wastewater have involved the assessment of disinfection efficiency, those factors affecting this efficiency and the outlining of some of the problems with the application of UV irradiation. Laboratory studies with UV light have demonstrated an adequate reduction in indicator bacteria from secondary effluents[6,7,8]. UV irradiated effluent exhibited no toxicity to fish when compared to undisinfected wastewater[3,8]. Jolley et al.[9] showed that UV irradiation of nonvolatile organics in secondary effluent produced only slight chemical changes in the nonvolatile organics while in one effluent mutagenic constituents were eliminated.

Pilot[10,11,12,13,14] and full scale[15] studies of the UV disinfection of sewage effluent have demonstrated that the process can consistently achieve the objective of 200 fecal coliforms per 100 ml of effluent. UV devices designed for disinfection of potable water were employed in most of these studies.

This project was initiated to assess all the operational aspects, and to monitor the efficiency of a full-scale ultraviolet device which was designed specifically for disinfecting wastewater.

MATERIALS AND METHODS

Project Site Description

The facility chosen for this study is located in Tillsonburg, Ontario, Canada. It is a conventional secondary plant which produces an above average effluent from primarily domestic sewage (Figure 1). The design capacity of the plant is 8.2×10^3 m^3/d with an average yearly flow of 4.6×10^3 m^3/d. Separate clarifiers, effluent channels and chlorine contact chambers allowed the simultaneous study of UV and chlorine disinfection.

Description of UV Unit

A modular system was designed to provide low maintenance and remain versatile. As shown in Figure 2, the system consists of a series of modules each of which is four quartz sheath enclosed UV lamps (13.8 W of 253.7 nm wavelength light, 76.2 cm arc length/lamp). Two sets of 10 modules for a total of 80 lamps are located in one effluent channel and are hereon described as Unit A. The second channel is 40 cm wider and contains 2 sets of 15 modules for a total of 120 lamps and is hereon called Unit B. The average flow over the lamps of Units A and B are 0.03 m^3/sec and 0.02 m^3/sec, respectively. Pumps and related maintenance are obviated because it is a gravity flow system. All of the major components are modular thereby eliminating a major shutdown if one component requires maintenance or repairing.

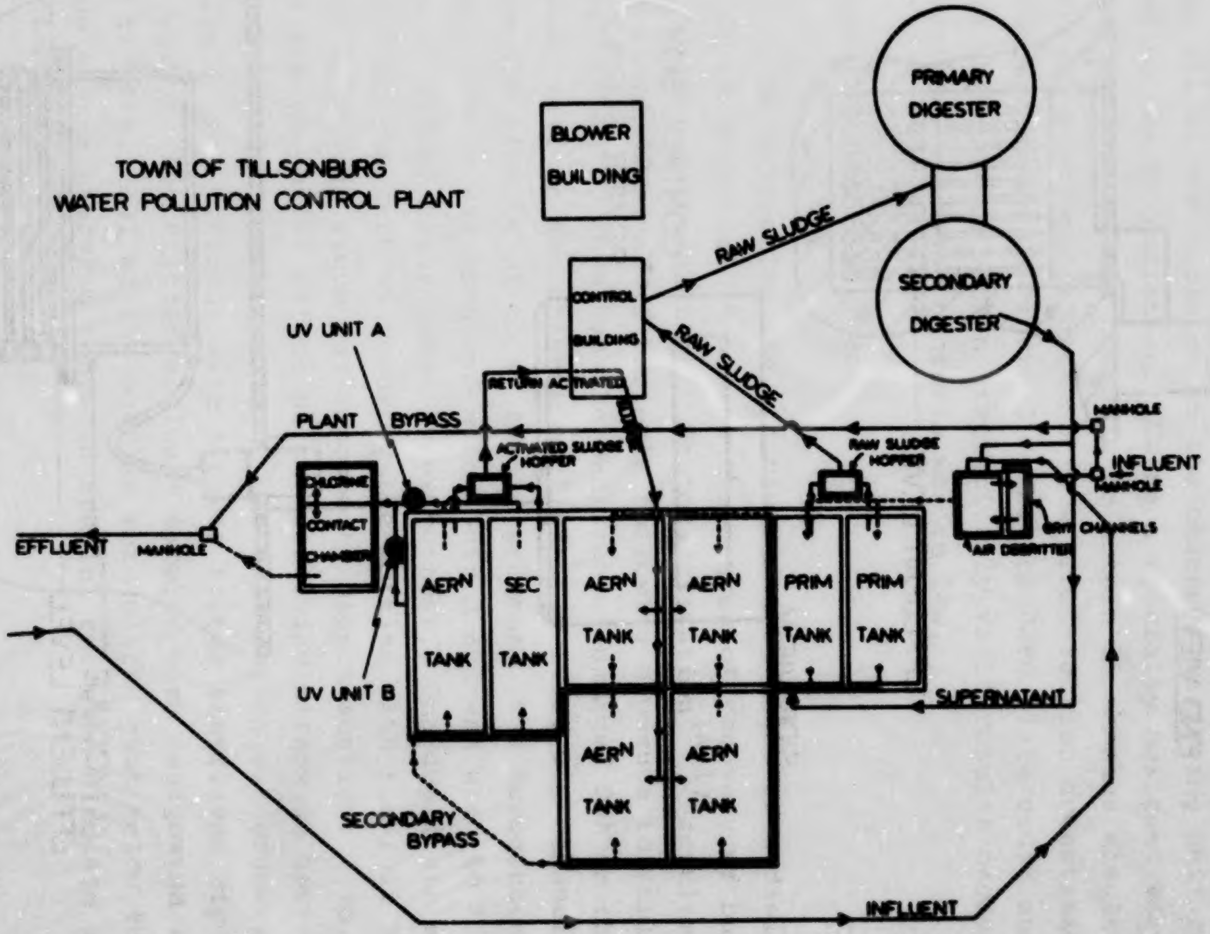

TOWN OF TILLSONBURG
WATER POLLUTION CONTROL PLANT
BLOWER BUILDING
PRIMARY DIGESTER
SECONDARY DIGESTER
RAW SLUDGE
CONTROL BUILDING
RAW SLUDGE
UV UNIT A
RETURN ACTIVATED
PLANT BYPASS
ACTIVATED SLUDGE HOPPER
RAW SLUDGE HOPPER
MANHOLE
INFLUENT
MANHOLE
GRIT CHANNELS
AIR DEGRITTER
CHLORINE CONTACT CHAMBER
EFFLUENT
MANHOLE
AERN TANK
SEC TANK
AERN TANK
AERN TANK
PRIM TANK
PRIM TANK
AERN TANK
AERN TANK
UV UNIT B
SUPERNATANT
SECONDARY BYPASS
INFLUENT

Figure 1. Design of the water pollution control plant.

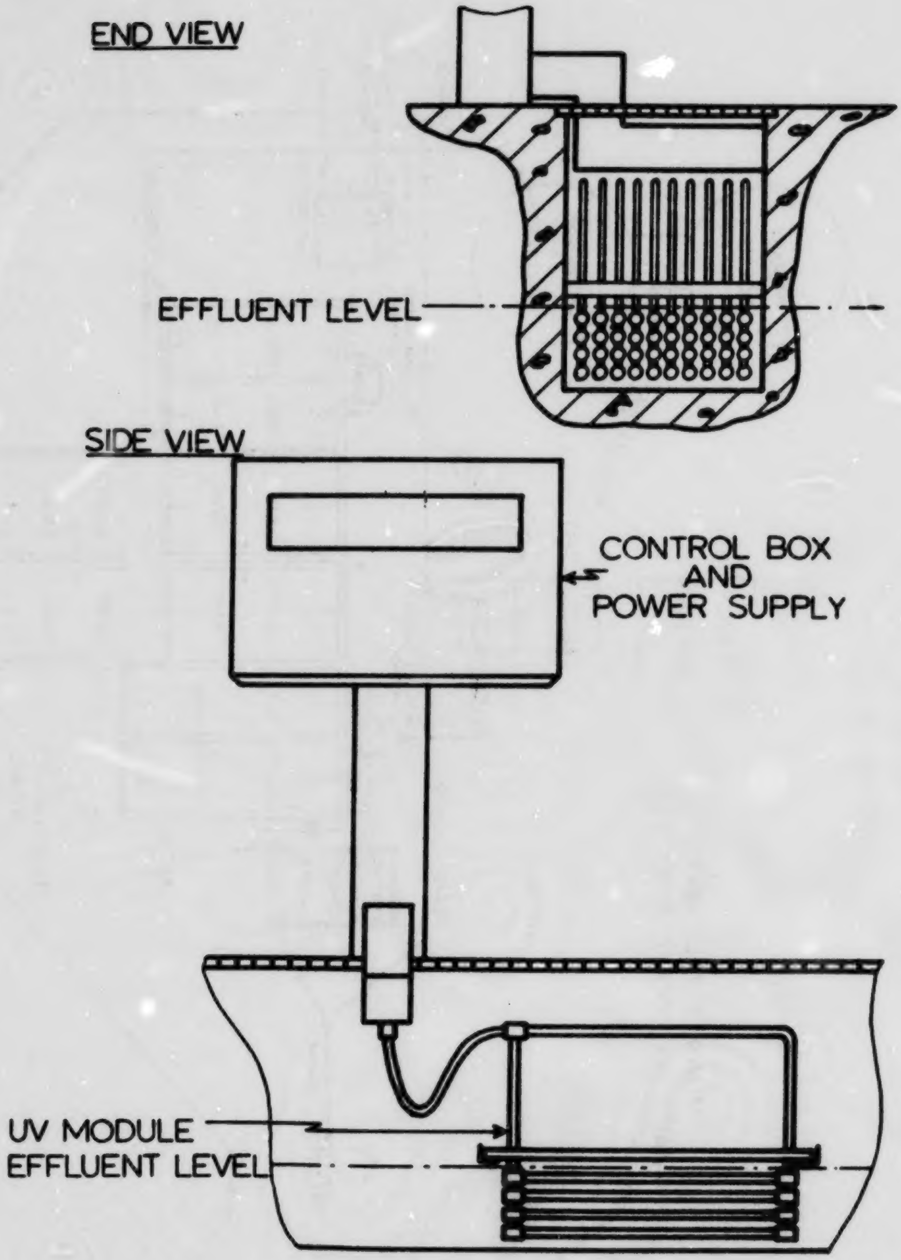

END VIEW
EFFLUENT LEVEL
SIDE VIEW
CONTROL BOX AND POWER SUPPLY
UV MODULE
EFFLUENT LEVEL

Figure 2. Schematic of the ultraviolet disinfection unit in the sewage treatment plant effluent channels.

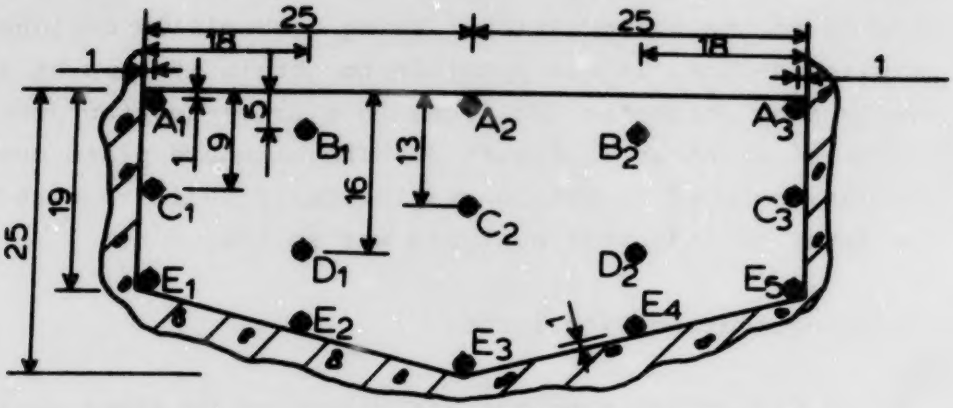
Uniform Exposure Experiment

A cross-sectional sampling of the effluents was conducted immediately downstream of Units A and B to determine whether all of the effluent in the channels was being uniformly exposed to the UV radiation. Using a specially designed vacuum sampling device, it was possible to obtain 15 samples simultaneously from strategic locations in a cross-section downstream of the units as shown in Figure 3. The standard plate count analysis was employed to obtain statistically valid results because the level of indicator bacteria was so low.

Photoreactivation Experiment

In evaluating the efficiency of UV light as a disinfectant, it is essential to assess the degree of recovery of bacteria following irradiation. Photoreactivation[16,17] results in an increased survival of bacteria following exposure to sunlight and dark repair[17] involves the excision of damaged DNA in the bacterial cell in the absence of light. A study was conducted to examine the effects of these repair systems. One homogeneous effluent sample was obtained after Unit A and was used to fill a series of 500 ml clear round Pyrex bottles. One duplicate series was kept in the dark by covering the bottles with tin-foil and another identical group was exposed to direct sunlight. To assess the potential effect of the receiving stream on bacterial recovery another duplicate light and dark series was prepared by diluting 1 part effluent with 3 parts filter sterilized Big Otter Creek water. The diluted effluent samples were designated 25% light or dark. All of the bottles were placed just below the surface of the settling tanks in special racks to simulate actual conditions.

CROSS SECTION OF UV UNIT A


25
25
18
18
1
1
5
9
13
16
19
25
1
1
A1
A2
A3
B1
B2
C1
C2
C3
D1
D2
E1
E2
E3
E4
E5

ALL DIMENSIONS ARE IN CENTIMETERS
SAMPLING POINTS ◆

CROSS-SECTION OF UV UNIT B

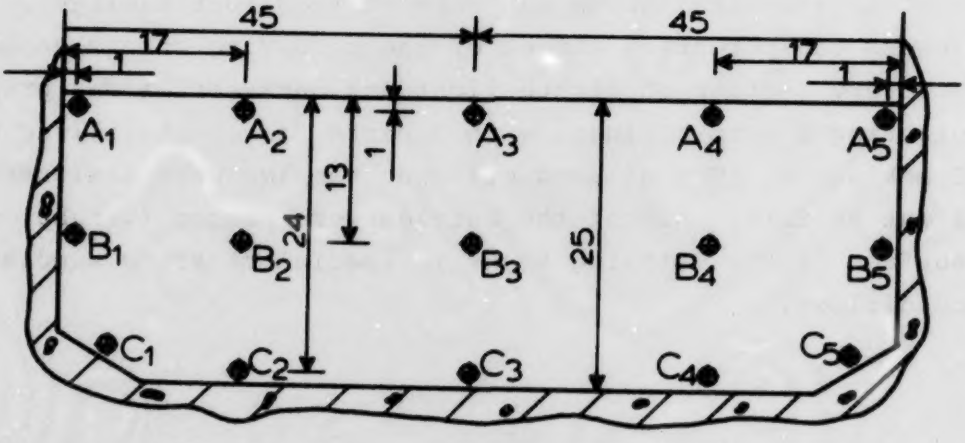

45
45
17
17
1
1
1
13
24
25
A1
A2
A3
A4
A5
B1
B2
B3
B4
B5
C1
C2
C3
C4
C5

Figure 3. Cross-sections of the channels showing the 15 sampling points.

Routine Sampling

As of July 1982, chemical and bacteriological sampling was carried out on a routine basis on the influents, between the matrices and effluents following Units A and B and the chlorine contact chamber.

Unit B was turned off 18h before sampling to allow the chlorine contact chamber to equilibrate to normal. Samples of chlorinated and UV disinfected effluent were exposed to the sunlight entering the clarifiers. Chlorine residuals were determined on the chlorinated effluent samples by the DPD method. All samples were immediately returned to the laboratory where the chemical and bacteriological analyses were conducted according to the Ontario Ministry of the Environment's Handbook of Analytical Methods[18].

Fish Toxicity Assay

A fish-toxicity evaluation was conducted in the receiving stream to compare the effects of UV and chlorine disinfection of the effluent. Rainbow trout yearlings were exposed in cages above and below the outfall of the sewage treatment plant (Figure 4). A variety of chemical and microbiological parameters were measured in the plant and in the receiving stream during the exposures.

Assay of Bacteriophage to *Escherichia coli* C

The bacteriophage in the wastewater before and after UV and chlorine disinfection were enumerated to obtain an indication of the effect of disinfection on an organism which closely resembles enteric viruses which are much more difficult to enumerate. The MPN method of Kott[19] was modified by using the agar, broth and *Escherichia coli* C. host of Scott *et al.*[20] to enumerate the bacteriophage.

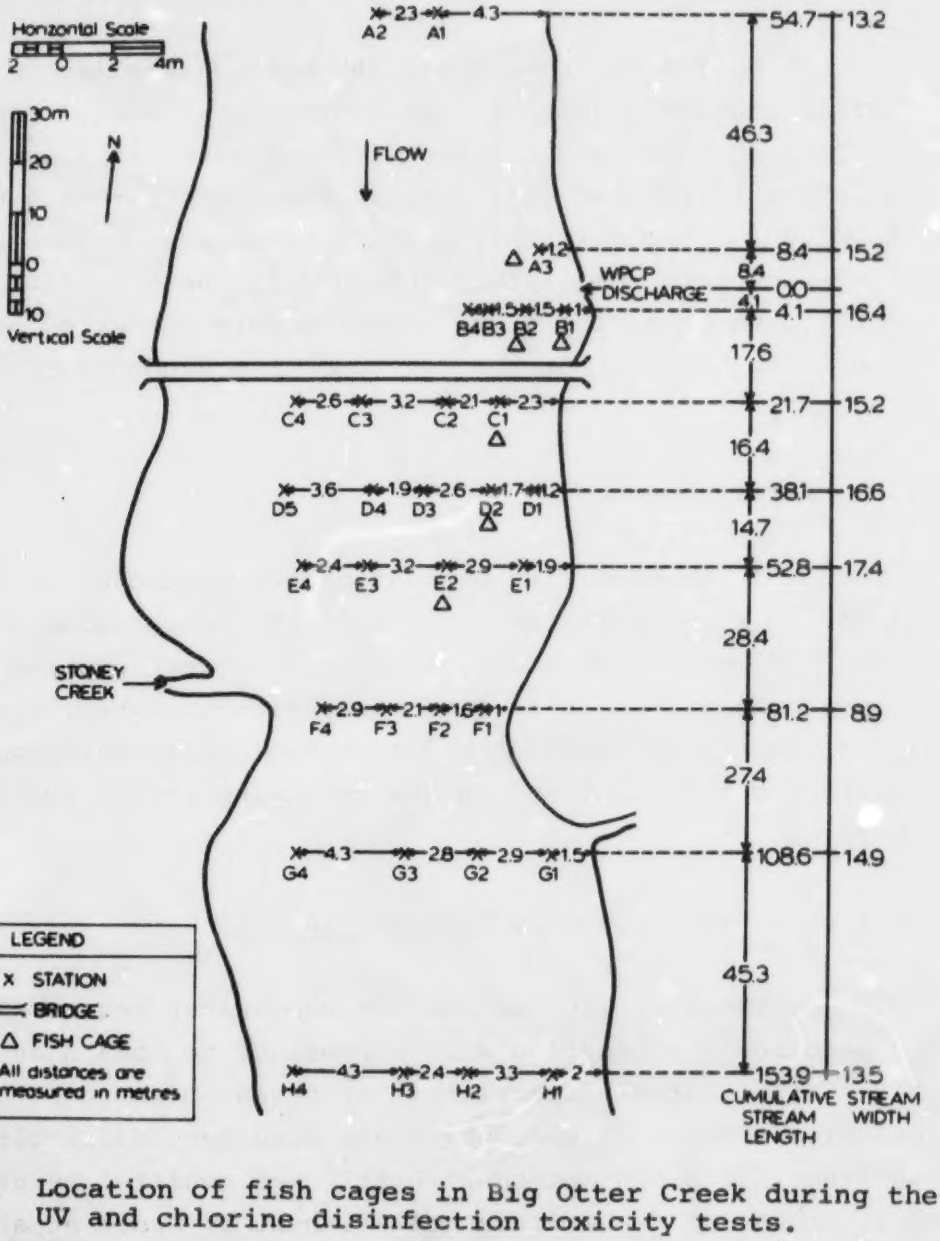

Horizontal Scale
2 0 2 4m
30m
20
10
0
10
Vertical Scale
N
FLOW
A2 23 A1 4.3
54.7 13.2
46.3
A3 1.2
8.4 15.2
8.4
WPCP DISCHARGE
0.0
4.1
B4 B3 1.5 B2 1.5 B1 1
4.1 16.4
17.6
C4 2.6 C3 3.2 C2 2.1 C1 2.3
21.7 15.2
16.4
D5 3.6 D4 1.9 D3 2.6 D2 1.7 D1 1.2
38.1 16.6
14.7
E4 2.4 E3 3.2 E2 2.9 E1 1.9
52.8 17.4
28.4
STONEY CREEK
F4 2.9 F3 2.1 F2 1.6 F1 1
81.2 8.9
27.4
G4 4.3 G3 2.8 G2 2.9 G1 1.5
108.6 14.9
45.3
H4 4.3 H3 2.4 H2 3.3 H1 2
153.9 13.5
CUMULATIVE STREAM LENGTH
STREAM WIDTH
LEGEND
X STATION
BRIDGE
△ FISH CAGE
All distances are measured in metres

Figure 4. Location of fish cages in Big Otter Creek during the UV and chlorine disinfection toxicity tests.

Enumeration of Clostridium perfringens

The following procedure is used for the examination of C. perfringens, a spore-forming bacterium which is known for its disinfection resistance[21]. A sample of wastewater is filtered through a 0.45 um filter (Type GN-6, Gelman Sciences Inc.). The membrane is then inverted, placed on C. perfringens agar for 1 minute after which the membrane is removed and discarded. The C. perfringens agar plate is incubated anaerobically at 44.5°C for 24 h. Lactose fermenting and lecithinase positive colonies are considered to be typical of C. perfringens.

To differentiate the vegetative cells of C. perfringens from those in the spore form, samples were heated at 80°C for 10 min and then rapidly cooled to room temperature with cold water. The samples were then analyzed as described previously. Unheated controls were also included.

Pathogen Testing

The effect of UV disinfection on waterborne pathogens in wastewater is unknown. Therefore, the UV and chlorinated effluents were tested for the following pathogens.

Pseudomonas aeruginosa was enumerated by a membrane filter technique according to the Ontario Ministry of the Environment's Handbook of Analytical Methods[18].

The level of Yersinia enterocolitica was determined by the method of Schiemann[22].

The level of Campylobacter jejuni was estimated by the modified methods of Doyle and Roman[23] and Blaser et al[24].

Salmonella species were detected using either a qualitative method which was that of Palmateer and Koellner[25], or a quantitative method. The quantitative analysis was a most probable number technique which included the filtration of approximately 10 l of sample using a sterile glass-fibre pre-filter and bacterial membrane filters of .45 um pore size. Nine samples of 3 separate 3-l, 300 ml and 30 ml aliquots were filtered after which each pre-filter and bacterial filter was placed in separate pre-enrichment broth labelled appropriately as to aliquot volume and source. At this point, further analysis is identical to that described above.

Cost Comparison

A cost comparison was prepared between the capital and operating costs of chlorination, dechlorination, chlorination-dechlorination, ozonation and UV disinfection by using the Innovative and Alternative Technology Assessment Manual of the EPA[26], the information from the UV disinfection project and data from Trojan Technologies Inc., London, Canada which manufactured the UV disinfection system. The costs in the Assessment Manual were updated to the 3rd quarter of 1982 by using the appropriate Engineering News Record Indexes. The amortization time is taken as 15 years and the interest rate is 12% semi-annually. The costing figures are reported as ratios to simplify the comparison between the various modes of disinfection.

RESULTS AND DISCUSSION

Uniform Exposure Experiment

As shown in Table 1, the geometric means of the standard plate count bacteria after 3 sets of samplings in both channels indicate an absence of short-circuiting of bacteria through the UV Units. This data showed that sampling at any location downstream of the UV Units would be representative of the degree of bacterial reduction.

Table 1. Results of the standard plate count of the cross-section sampling of Units A and B.

Location	Unit A Bacteria per ml Sampling 1	2	3	GM*	Location	Unit B Bacteria per ml Sampling 1	2	3	GM*
A1	6	4	16	7	A1	2	11	3	4
A2	6	4	4	5	A2	2	2	1	2
A3	5	4	35	9	A3	2	3	0	1
B1	5	3	3	4	A4	3	2	1	2
B2	6	3	4	4	A5	3	8	1	3
C1	4	3	2	3	B1	1	4	0	1
C2	4	5	2	3	B2	2	1	1	1
C3	6	8	3	5	B3	2	2	1	2
D1	12	4	3	5	B4	2	1	0	1
D2	5	3	3	4	B5	2	137	1	6
E1	6	6	3	5	C1	3	1	1	1
E2	5	5	5	5	C2	3	3	6	4
E3	6	13	6	8	C3	2	3	4	3
E4	6	6	4	5	C4	1	2	1	1
E5	3	5	4	4	C5	3	12	3	5
Influent	2500	5700	2800	3417	Influent	2400	3400	3300	2997

*GM - Geometric Mean

Photoreactivation Experimentation

The data in Figure 5 shows the means of four separate photoreactivation experiments. Total coliform and fecal coliform results indicate a maximum increase of 1 logarithm whereas the fecal Streptococci failed to photoreactivate after 6h exposure to sunlight. A dark repair mechanism may have existed but it was undetectable in this data. Mixing the irradiated effluent with sterile Big Otter Creek water did not enhance the repair mechanisms after 6h. Conversely, any potential toxicity of the irradiated effluent to the indicator bacteria was not detectable in the samples diluted with creek water. Although the results were somewhat variable, a 3h exposure appeared to be optimal for the routine sampling study. A full scale study by Scheible and Bassell[15] showed that a 1h exposure was adequate for optimal photoreactivation. Factors such as disinfection efficiency and the specific nature of the wastewater being treated may account for the difference.

Routine Monitoring

The results of the bacteriological analysis of samples taken before, in between the two sets of UV modules in each unit and after UV Units A and B are shown in Tables 2 and 3. As can be seen in these tables, the geometric mean and the range of the total and fecal coliforms in the effluent following UV disinfection and subsequent photoreactivation are well below the suggested Ontario Ministry of the Environment Guidelines of 2500 total and 200 fecal coliforms per 100 ml of effluent. In between the two sets of UV modules of Units A and B the geometric mean of the total and fecal coliform numbers is below the suggested guidelines before and after phototreactivation but the range of the coliform numbers exceeds the limit. Therefore, both sets of UV modules are required if the coliform numbers are to be kept below the guidelines at all times. The average log increase in the total coliform numbers after 3 h photoreactivation was 0.99

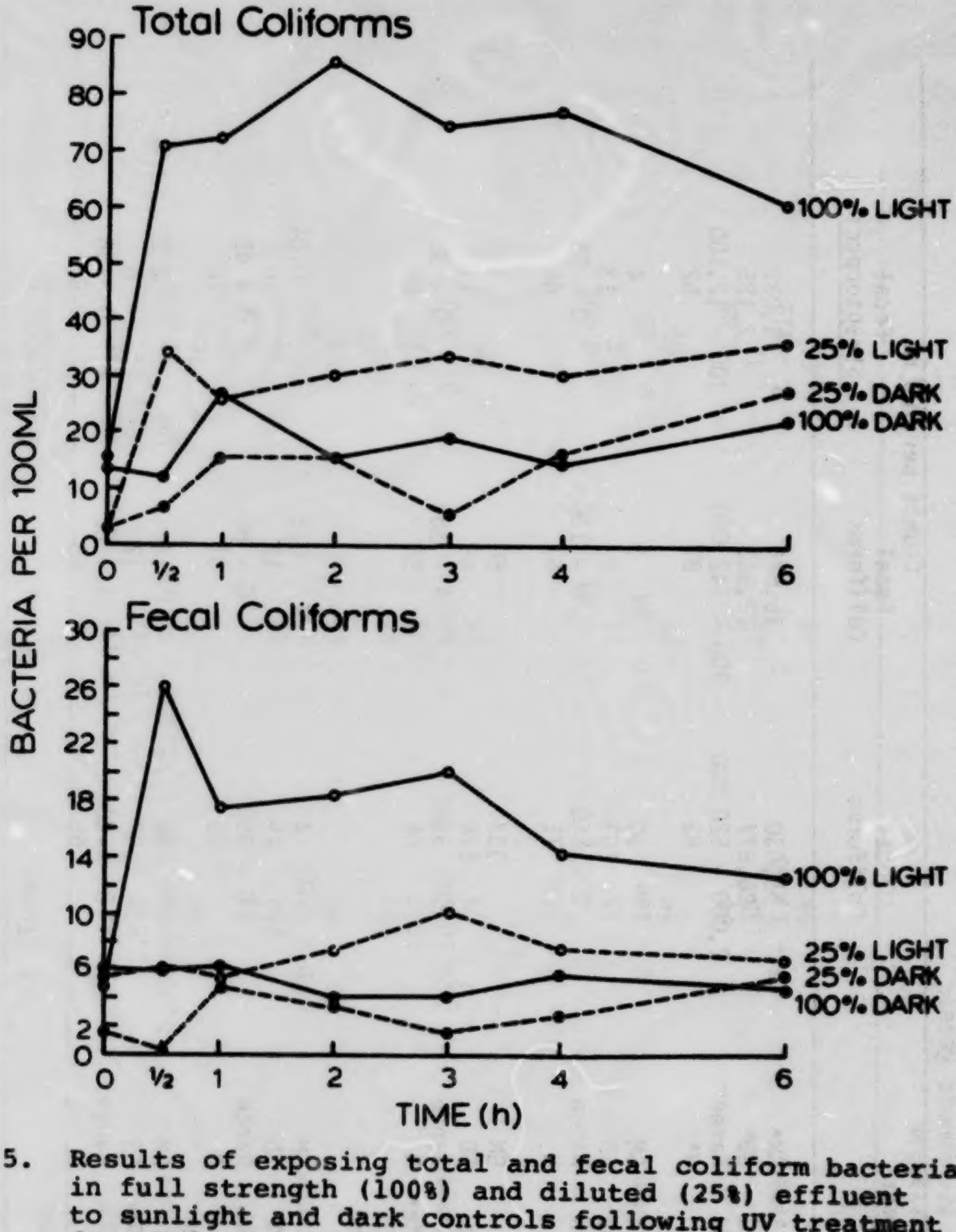


Figure 5. Results of exposing total and fecal coliform bacteria in full strength (100%) and diluted (25%) effluent to sunlight and dark controls following UV treatment from Unit A.

Table 2. Results of the routine bacteriological analysis of the wastewater before, in between the two sets of UV modules and after UV Unit A.

Sample Source		Counts per 100 ml		
		Total Coliforms	Fecal Coliforms	Fecal Streptococci
Influent to Unit A	GM*	53,030	15,645	1,239
	SD*	86,877	22,363	2,125
	Range*	2,000 - 520,000	900 - 142,000	100 - 12,100
	N*	83	87	82
Effluent Between the Sets of UV Modules	GM	30	5	4
	SD	103	23	13
	Range	0 - 560	0 - 136	0 - 55
	N	71	67	66
Effluent Between the Set of UV Modules after 3 h Photo-reactivation	GM	337	50	4
	SD	676	49	11
	Range	32 - 3900	4 - 188	0 - 38
	N	56	55	56
Effluent from Unit A	GM	4	0.3	0.04
	SD	49	12	6
	Range	0 - 380	0 - 86	0 - 42
	N	75	81	76
Effluent from Unit A After 3h Photo-reactivation	GM	66	14	0.2
	SD	87	17	6
	Range	8 - 420	1 - 82	0 - 36
	N	65	65	62

*GM - Geometric Mean
SD - Standard Deviation
Range - Highest and Lowest Result
N - Number of Samples

Table 3. Results of the routine bacteriological analysis of the wastewater before, in between the two sets of UV modules and after UV Unit B.

Sample Source		Counts per 100 ml		
		Total Coliforms	Fecal Coliforms	Fecal Streptococci
Influent to Unit B	GM*	39,447	7,636	598
	SD*	72,816	16,447	2,227
	Range*	6,000 - 490,000	1,000 - 92,000	140 - 17,400
	N*	66	73	70
Effluent Between the Sets of UV Modules	GM	35	6	0.9
	SD	294	49	28
	Range	0 - 1500	0 - 288	0 - 150
	N	59	61	59
Effluent Between the Sets of UV Modules after 3 hr Photo-reactivation	GM	291	57	1.2
	SD	1637	132	27
	Range	10-10,300	3 - 600	0 - 150
	N	53	54	54
Effluent from Unit B	GM	4	0.01	0.002
	SD	33	11	5
	Range	0 - 198	0 - 64	0 - 25
	N	64	72	69
Effluent from Unit B After 3h Photo-reactivation	GM	31	7	0.05
	SD	138	15	16
	Range	3 - 1,000	0 - 57	0 - 119
	N	58	59	58

*GM - Geometric Mean
SD - Standard Deviation
Range - Highest and Lowest Result
N - Number of Samples

and 1.08 respectively in between the 2 sets of UV modules in each unit and after the UV units. This is in agreement with the results of Scheible and Bassell[15] but not Bohm et al[28] who found an increase of 1.4 to 1.7 orders. The UV energy fluence had no effect on the degree of photoreactivation as it remained almost identical in between the UV modules of each unit and after the units. The fecal Streptococci were reduced substantially by the UV units and remained low as this group failed to photoreactivate as was reported by others[14]. Table 4 shows that the indicator bacterial levels in the chlorinated effluent are similar to the levels found in the UV irradiated effluent following photoreactivation. The chemical analyses (Table 5) conducted on the effluents at the time of bacterial analyses indicated a good quality effluent, which was free of any toxic chemicals which would have complicated any analyses during this study.

Fish Toxicity Assay

Results of chemical analyses conducted on the effluent on site during the toxicity assay are shown in Table 6. During the chlorination study, the effective time to death for 50% of the test fish was 7h from the test initiation at all downstream stations and complete mortality occurred within 24h. During an initial exposure to UV disinfected effluent, a few fish mortalities were observed (maximum of 14% at any one site). It was attributed to primary sewage which bypassed the plant treatment system during a storm event. A repeat UV disinfection test was non-lethal for a 48h exposure period.

Bacteriophage Assay

The results in Table 7 show that Units A and B killed 99.97 and 99.98% respectively of the bacteriophages whereas chlorination killed 95.1%. This indicates that UV irradiation may be more effective at reducing the level of enteric viruses in wastewater.

Table 4. Results of the routine bacteriological analysis of the effluent before and after chlorine disinfection.

Sample Source		Counts per 100 ml Total Coliforms	 Fecal Coliforms	 Fecal Streptococci
Influent to the Chlorine Contact Chamber	GM*	40,827	7,203	578
	SD*	78,928	12,600	2,203
	Range*	6,000 - 490,000	84 - 81,000	140 - 17,400
	N*	55	60	60
Effluent from the Chlorine Contact Chamber	GM	101	0.4	14
	SD	4,482	205	56
	Range	0 - 21,800	0 - 1,500	0 - 286
	N	56	61	61
Effluent from the Chlorine Contact Chamber after 3h Exposure to Sunlight	GM	98	4	0.2
	SD	959	62	56
	Range	10 - 6,700	0 - 310	0 - 340
	N	54	55	55

*GM - Geometric Mean
SD - Standard Deviation
Range - Highest and Lowest Result
N - Number of Samples

Table 5. Means of the measured chemical parameters from July 1982 to September 1983

Parameters	Influent to Unit A	Influent to Unit B	Effluent from Chlorine Contact Chamber
Flow Rate 10^3 x m^3/d	3.7	2.9	
Chlorine residual mg/l			0.4
%T*, 254 nm, 1 cm	77.9	77.5	77.9
%T, 365 nm, 1 cm	94.6	94.3	94.5
Dissolved organic carbon mg/l	4.2	4.3	4.5
Apparent colour in Hazen Units	18.4	17.9	15.4
Turbidity in Formazin Units	1.9	1.8	2.2
pH	7.1	7.1	7.1
Conductivity in umho/cm^3 at 25°C	1011.3	986.8	997.6
Iron as Fe mg/l	0.037	0.027	0.032
Nitrogen mg/l			
(1) Free ammonia	0.4	0.6	0.8
(2) Total Kjeldahl	1.2	1.4	1.9
(3) Nitrite	0.3	0.3	0.3
(4) Nitrate	18.3	17.1	17.1
Phosphorus as P			
(1) Total mg/l	0.62	0.57	0.57
(2) Dissolved reactive mg/l	0.21	0.19	0.19
BOD mg/l	2.8	2.4	2.1
Suspended solids mg/l	6.1	5.3	5.6
Dissolved solids mg/l	766.5	653.8	648.2
COD	14.6	14.6	14.3

*%T - Percent Transmission

Table 6. Chemical parameters measured on site during the fish toxicity study.

	UV Run 1	UV Run 2	Chlorine Run
Chlorine Residuals			
(1) Total Chlorine Residual ug/l	0	0	178 - 1,510
(2) Monchlaromine ug/l	0	0	92 - 1,080
(3) Dichloramine ug/l	0	0	128 - 600
Nitrogen mg/l			
(1) Free ammonia	0.048 - 4.500	0.002 - 1.02	0.020 - 0.950
(2) Total Kjeldahl	0.69 - 1.63	0.63 - 1.30	0.63 - 2.65
pH	7.20 - 8.20	7.24 - 7.47	7.09 - 7.48
Conductivity umho/cm³ at 25°C	463 - 1,039	940 - 1,170	825 - 1,130
Phenols ug/l	*L0.2 - 8.8	*N.D.	1.2 - 2.4

*N.D. - Not Done
L - Less Than

Table 7. Results of the MPN Method of counting bacteriophages to E. coli C before and after UV and chlorine disinfection.

Sample Source	Bacteriophage counts per 100 ml Geometric Mean	Standard Deviation	Number of Samples
Influent to Unit A	3,530	12,568	12
Effluent from Unit A	0.95	4.62	12
Influent to Unit B and the Chlorine Contact Chamber	6,233	40,898	12
Effluent from Unit B	1.4	3.4	11
Effluent from the Chlorine Contact Chamber	304	3,205	11

Table 8. Results of the routine enumeration of *C. perfringens*

Sample Source	Counts per 100 ml			Number of Samples
	Geometric Mean	Standard Deviation	Range	
Influent to Unit A	1,854	1,296	100 - 9,000	71
Effluent Between the Two Sets of UV Modules in Unit A	618	958	0 - 3,900	56
Effluent Between the Two Sets of UV Modules in Unit A after 3h Photoreactivation	801	936	100 - 3,600	51
Effluent from Unit A	358	626	16 - 2,600	66
Effluent from Unit A After 3h Photoreactivation	323	349	10 - 1,900	56
Influent to Unit B	1,625	1,174	248 - 5,900	62
Effluent Between the Two Sets of UV Modules in Unit B	402	890	8 - 3,700	51
Effluent Between the Two Sets of UV Modules in Unit B after 3h Photoreactivation	249	803	0 - 3,100	51
Effluent from Unit B	27	205	0 - 830	61
Effluent from Unit B after 3h Photoreactivation	81	204	2 - 850	54
Influent to the Chlorine Contact Chamber	1,683	1,056	500 - 5,400	54
Effluent from the Chlorine Contact Chamber	1,292	1,068	76 - 4,500	55
Effluent from the Chlorine Contact Chamber after 3h Exposure to Sunlight	814	1,147	0 - 5,300	50

Enumeration of *C. perfringens*

The routine results from Table 8 show that Units A and B killed approximately 1.8 times as many *C. perfringens* as chlorination. Based on the differentiation of vegetative cells and spores, neither UV or chlorine appeared to affect the spores.

Pathogen Testing

As shown in Table 9, UV and chlorine disinfection were both equally effective in destroying *Pseudomonas aeruginosa*.

In two preliminary runs, *Yersinia enterocolitica* was reduced equally by both modes of disinfection. However, due to the sensitivity of the bacterium to warm waters, further analysis will commence when the temperature of the effluent is below 10°C[27].

Using the most current isolation methods, *Campylobacter jejuni* was not detected in the undisinfected wastewater.

The quantitative analysis revealed that UV and chlorine were equally efficient at reducing the *Salmonella* population. The qualitative method showed that *Salmonella sp* were present in the UV irradiated effluent but not in the chlorinated effluent as detected by a Moore swab suspended in the effluents for 24-96 h. The absence of *Salmonella* in the chlorinated effluent may be due to the residual of chlorine which continuously bathed the swabs.

Table 9. Results of the analysis for Pseudomonas aeruginosa in the effluents before and after UV and chlorine disinfection

Sample Source	Counts per 100 ml Geometric Mean	Standard Deviation	Range	Number of Samples
Influent to Unit A	151	321	24 - 1500	26
Effluent from Unit A	1	0.3	*L4 - 0	25
Effluent from Unit A after 3h Photoreactivation	1.1	0.75	3 - 0	20
Influent to Unit B and the Chlorine Contact Chamber	153	413	16 - 1500	23
Effluent from Unit B	1.0	0.46	2 - 0	22
Effluent from Unit B after 3h Photoreactivation	1.0	0.4	L4 - 0	19
Effluent from the Chlorine Contact Chamber	1.4	21	0 - 101	22
Effluent from the Chlorine Contact Chamber after 3h Exposure to Sunlight	1.3	7.3	32 - 0	19

*L - Less Than

Cost Comparison

The ratios in Table 10 show that UV disinfection can be a cost effective alternative to other modes of disinfection. UV lamp replacement and power usage make up the majority of the UV operating costs when the amortization costs are excluded. The lamp replacement time has been taken as 18 months because in this study the UV lamps have operated consistently for 18 months. Ozonation is not cost competitive with UV irradiation because ozonation has high installation and operating costs.

Maintenance Observations

Although current results have remained consistently satisfactory, the UV lamps have been cleaned once. During 18 months of total operation, no masses of algae have been observed to build up on the units because the UV lamps are parallel to the flow. Five UV lamps have been replaced during the 18 months.

CONCLUSIONS

1. These results show that UV disinfection of treated wastewater is a viable alternative to chlorination if the UV unit is designed specifically for this purpose.
2. This study demonstrated that UV devices can be designed which require minimal maintenance and very little on-site modifications.
3. UV irradiation of wastewater can reduce the bacterial concentrations to safe levels without introducing a toxicant to fish in the receiving waters.

Table 10. Comparision of capital and operating costs for chlorination, dechlorination, chlorination-dechlorination, ozonation and ultraviolet disinfection in the United States

	Capital Cost Ratios Plant Size (10^3 m^3/d)			
Disinfection Mode	3.78	18.9	37.8	378
Chlorination	2.01	1.76	1.45	0.86
Dechlorination	0.68	0.48	0.36	0.14
Chlorination-Dechloration	2.69	2.24	1.81	1.00
Ozonation by air	9.98	7.46	7.27	6.96
UV (Trojan UV2000 System)	1	1	1	1
	Operation Cost Ratios			
Chlorination	2.77	2.46	2.11	1.47
Dechlorination	1.19	0.90	0.72	0.41
Chlorination-Dechlorination	3.96	3.36	2.84	1.88
Ozonation by air	8.67	6.87	6.73	7.05
UV (Trojan UV2000 System)	1	1	1	1

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FIELD MEASUREMENT OF INFILTRATION THROUGH LANDFILL COVERS

BY

P.K. LEE, GARTNER LEE ASSOCIATES LIMTED
A.G. HIMS, GARTNER LEE ASSOCIATES LIMITED
DR. R. GILHAM, UNIVERSITY OF WATERLOO

ABSTRACT:

The usual source of landfill leachate is related to the infiltration of precipitiation through the soil cover and then refuse. The prediction of infiltration through landfill surface covers is often qualitative and is poorly documented. Minimal data are available to assess the effectiveness, reliability and design of current and past practices relative to leachate production. A two phased study, of which this is the first, is to be carried out over a three year period. The objectives of Phase I reported here are to design a suitable lysimeter applicable to a variety of landfill environments that will provide economical repeatable and accurate measurements. In Phase 2 a number of variables and settings will be documented. A literature search and review was the initial task undertaken and provided the basis for design. Installations were constructed at the Britannia Road Landfill in Mississauga in the Region of Peel. This site is an engineered facility with cells constructed and based in low permeability clay subsoils. The landfill is a containment site with provisions for leachate collection and off-site treatment. The final soil cover is a compacted silty clay. Two types of lysimeters are reported upon, one being of a sophisticated data accumulation type including a storage well and an automatic data recording mode while the other is a simple sheet lysimeter. The storage well lysimeter has met the objectives.

The paper presents details of both designs along with installation and operation aspects and problems. Data accumulated since March, 1983 are presented along with preliminary analysis and recommendations.

INTRODUCTION:

One of the major controlling parameters required to properly design solid waste disposal sites with regard to leachate collection aspects and impact predictions, is of course the rate of leachate generation. Where landfilling is carried out above the ground water table, leachate generation is directly proportional to the amount of infiltration of precipitation which occurs through the landfill cover material. Studies have been carried out in the United States, namely for the Environmental Protection Agency, dealing with the design and assessment of landfill covers. Present design practice utilizes both empirical values and agricultural drainage equations together with climatic factors, in order to predict likely infiltration volumes and thus leachate generation rates. No hard data are available for Ontario landfills except for a few studies that are of a general form. In the situations where leachate volumes have actually been measured, the rate of generation of leachate is invariably greater than that originally predicted.

The overall goal of the present project is to collect field measurements of the actual infiltration through various types of final cover materials, located in varying physical settings. The project is designed in two phases and is intended to be completed in approximately three years.

This paper reports on Phase 1 of the project, the main objective of which is to design and construct a field lysimeter installation that will provide economical, accurate and reproduceable measurements of infiltration at an existing landfill site.

LITERATURE REVIEW:

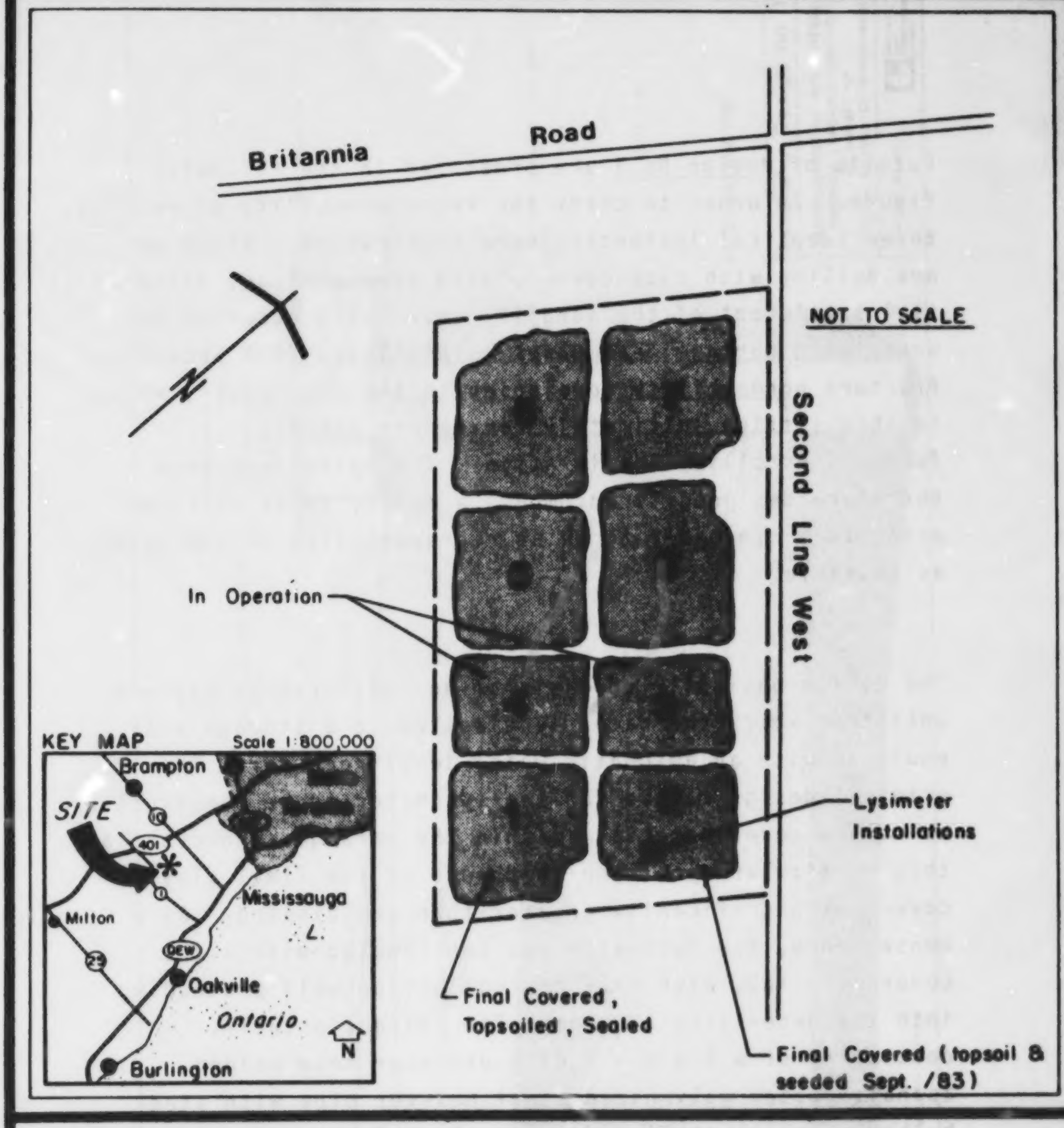
The first task of the project was to complete a literature review of past practices for measurement of infiltration in general and lysimeter installations in particular. A list of references is provided at the end of the paper. Most references encountered were concerned with the measurement or prediction of surface drainage or infiltration from the standpoint of either crop irrigation or ground water recharge. No references to the use of lysimeters for measurement of infiltration through landfill covers were found.

SITE LOCATION:

The site chosen for the installation of the lysimeters was the Central Britannia Road Landfill Site, Mississauga, located in the Region of Peel. This site is an engineered facility with cells constructed and bottomed in low permeability Halton clay till subsoils. The site layout is shown on the following figure. The landfill is a leachate containment site with provisions for full leachate collection by means of underdrains and off-site treatment. The final soil cover is a well compacted silty clay till, which is topsoiled and seeded.

LYSIMETER DESIGN:

Originally, only one lysimeter design was proposed, of a relatively sophisticated data accumulation type, that included a storage well and automatic data recording mode. Subsequent to undertaking the project, it was suggested by MOE staff that a much simpler type of sheet lysimeter should also be constructed and the results compared to those from the more sophisticated design.


Britannia Road
Second Line West
NOT TO SCALE
N
In Operation
Lysimeter Installations
Final Covered, Topsoiled, Sealed
Final Covered (topsoil & seeded Sept. /83)
KEY MAP
Scale 1:800,000
Brampton
SITE
10
401
Mississauga
Milton
25
QEW
Oakville
L.
Ontario
N
Burlington

BRITANNIA ROAD LANDFILL CELL LAYOUT

Fig. 1

MEASUREMENT OF INFILTRATION

Legend

— — Landfill Boundary

▨ Approximate Outline Of Cell

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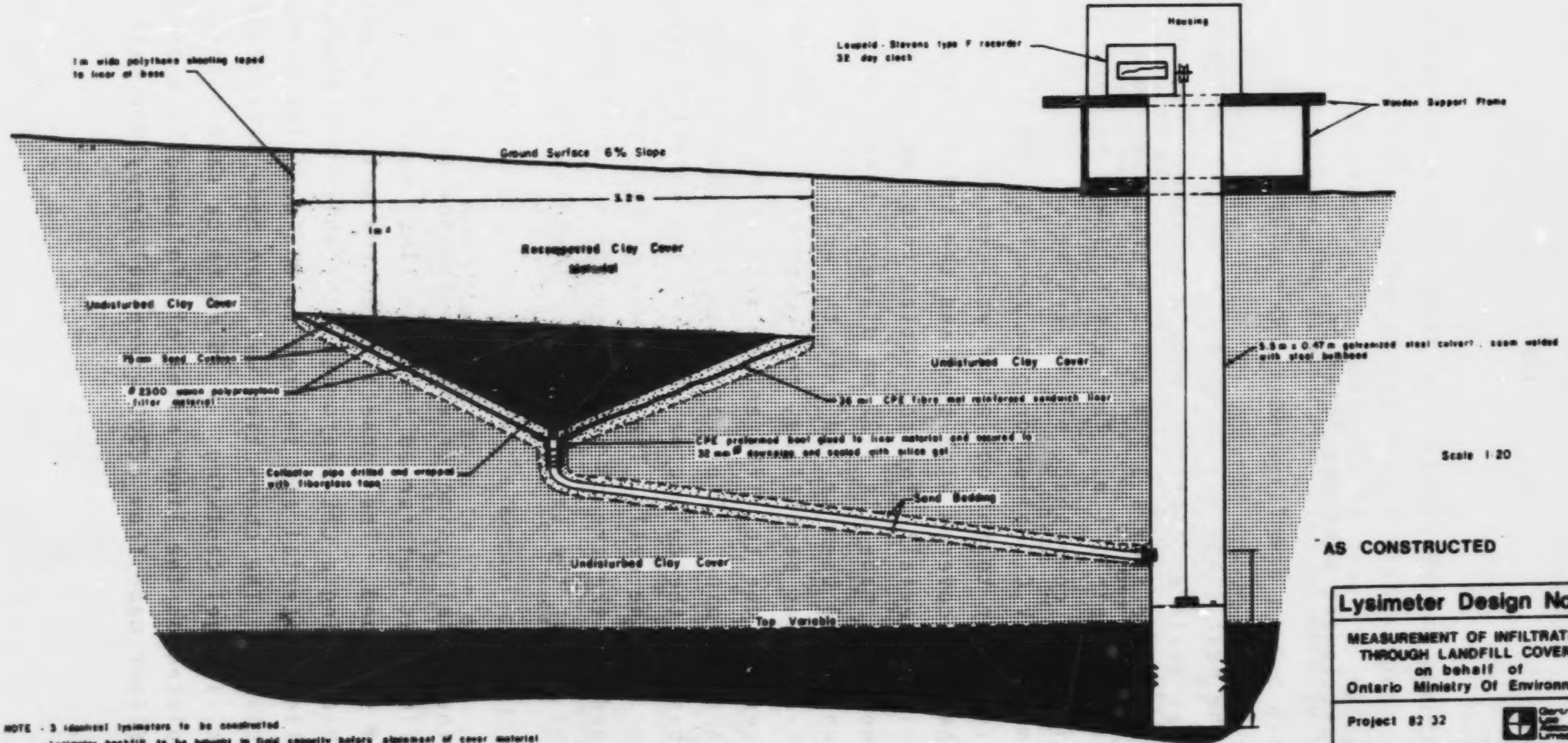
Details of design No.1 are presented in the following figure. In order to check the reproduceability of results, three identical lysimeters were constructed. Since we are dealing with clay cover placed over garbage, differential settlement of the landfill cover will occur as the underlying garbage decomposes. This leads to a secondary fracture permeability developing in the clay cover, which in this setting, ultimately can become the major factor controlling infiltration. The lyismeters were therefore designed to encompass a fairly large surface area, 10 m^2 in order to be as representative of the cover as possible.

The design basically consists of an infiltration capture unit from which the water is directed to a storage well equipped with an automatic water level recorder. The original design called for the lysimeter to be constructed below the cover material, within the garbage. However, at this particular site, the thickness of the final clay cover was significantly greater than anticipated. As a consequence, the lysimeter was constructed within the cover material, with only the collection well protruding into the underlying garbage. The collection well, consisting of a 5.5 m x 0.47 m diameter seam welded, asphalt coated galvanized steel hel-cor pipe with steel bulkhead, was installed first in late October 1982. The pipe was installed by means of a backhoe excavator because it was not possible to penetrate the garbage with a large diameter stirling auger drill rig, as was originally planned. The size of the excavation required in order to install the collection well and subsequent backfilling, did create some


Housing
Leupold - Stevens type F recorder
32 day clock
Wooden Support Frame
1m wide polythene sheeting taped
to liner at base
Ground Surface 6% Slope
3.2 m
1m ±
Recompacted Clay Cover
material
Undisturbed Clay Cover
75mm Sand Cushion
Ø 2300 woven polypropylene
filter material
36 mil CPE fibre mat reinforced sandwich liner
CPE preformed boot glued to liner material and secured to
32 mm Ø downpipe and sealed with silica gel
Collector pipe drilled and wrapped
with fiberglass tape
Sand Bedding
Undisturbed Clay Cover
Undisturbed Clay Cover
5.5 m x 0.47 m galvanized steel culvert, seam welded
with steel bulkhead
Top Variable

Scale 1:20

"AS CONSTRUCTED"

NOTE - 3 identical lysimeters to be constructed.
- Lysimeter backfill to be brought to field capacity before placement of cover material

Lysimeter Design No. 1

MEASUREMENT OF INFILTRATION
THROUGH LANDFILL COVERS
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settlement problems later on.

The next stage was to excavate for the lysimeter, which was again accomplished with a backhoe. The clay cover was excavated from a 3.2 m x 3.2 m area down to a depth of approximately 1 m. This was followed by excavation of the inverted cone shape of the lysimeter. The 32 mm diameter P.V.C. collector pipe running from the base of the lysimeter to the collector well was then placed on a sand bed and covered with sand and the trench backfilled with clay material. The connection into the collector well was made via a 32 mm diameter threaded female stub which had been welded into the well. The first time the trench was backfilled, the connection at the well severed due to differential settlement between the well and the pipe. The trench was re-excavated down to the base of the collector well and the material recompacted around the well. The pipe was re-laid on a thicker sand bed and the trench backfilled again. This problem occurred at two of the three lysimeter installations.

The acutal lysimeter was constructed using a 36 mil CPE fibre mat reinforced sandwich liner material placed on a sand bed. The connection to the collector well was made using a CPE preformed boot which was glued to the liner material and secured to the downpipe using screw clamps. The connection was then made watertight using silica gel caulking. A 75 mm thick sand cushion was placed on the liner material. followed by a protective filter made from #2300 woven polypropylene material.

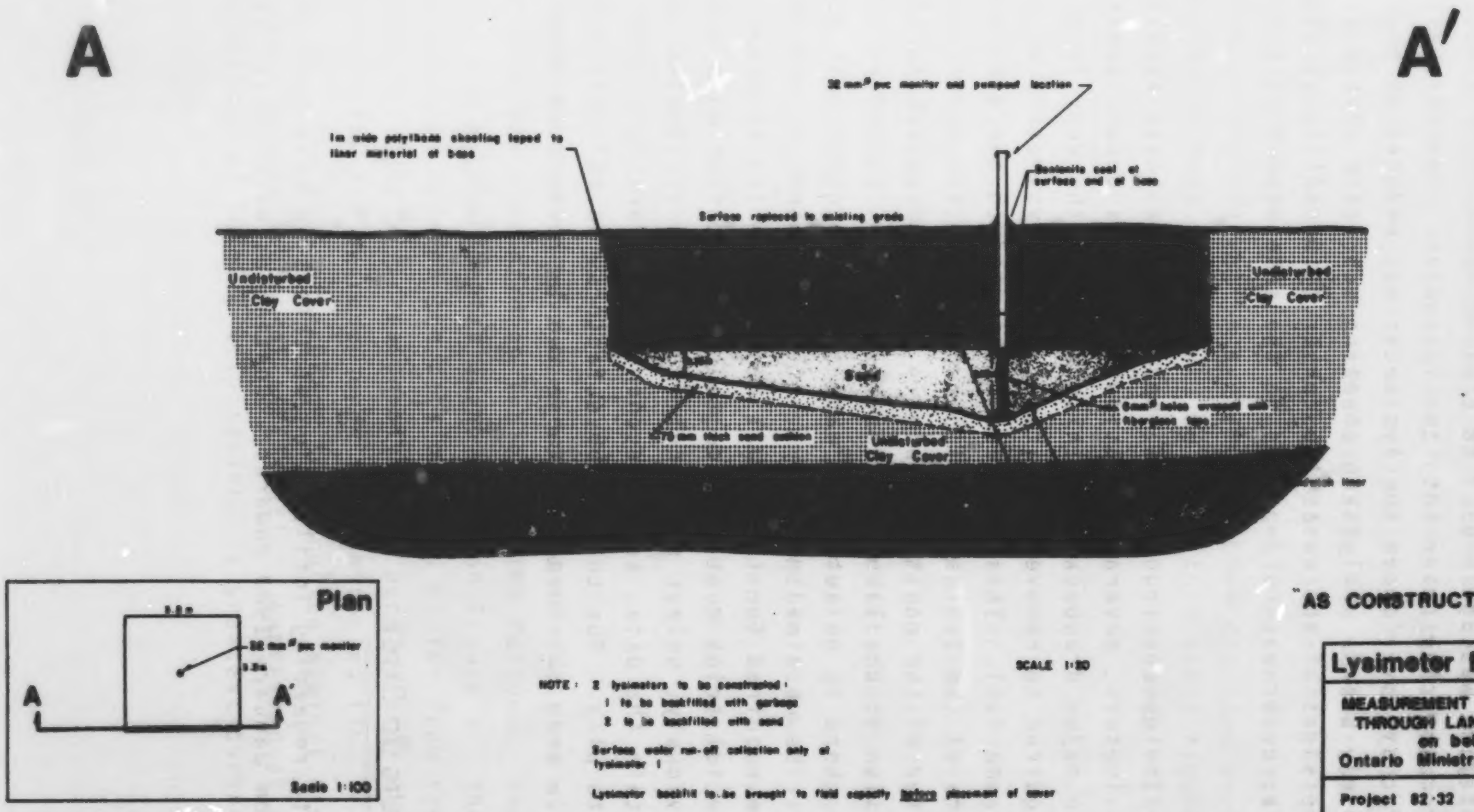
This was used as a precaution against accidental puncturing of the liner as the lysimeter was filled with garbage. Garbage was used as the fill material in order to reflect as close as possible, the actual situation of garbage beneath a clay cover. The filter cloth material was placed over the top of the garbage fill, to prevent downward migration of fines from the clay cover into the lysimeter. As construction of the three lysimeters was done concurrently, the excavations were open for in excess of one week. Several rainfall events occurred during this time which served to saturate the lysimeters and provide a check on the collection system and storage well.

Prior to replacing the clay cover material, polythene sheeting was placed around the full height of the excavation and attached to the lysimeter liner to prevent ingress of water through the sides of the excavation. The clay cover was then re-compacted into the excavation, in lifts of 150 mm using a gas-driven plate compactor. In situ density tests, which were performed on the clay cover material prior to construction in order to determine the existing conditions indicated an average density of 2 t/m^3. Further testing is to be carried out at each installation as a check on the actual compaction achieved. The clay cover was replaced to the existing grade. The slope of the surface at this location was approximately 6%.

The final stage of the construction was to install the Leupold-Stevens type F continuous chart water level recorders. The recorder housings were built to MOE specifications and supported on a wooden framework which also served as a surface support system for the collector wells The chart recorders, which are equipped with a battery operated, 32 day clock, have been operational since Mid-March 1983. Since it had been necessary to pump out water from the collection wells on several occasions, both during and after construction, it was assumed that the installations were at field capacity by the time the recorders were operative. Removal of water from the collection wells is accomplished by means of a contractors sump pump, equipped with a 6 m intake line. The ground surface at the installations remained without topsoil until the area was seeded in early September.

Details of lysimeter design No. 2 are presented in the following figure. The design consists of a simple sheet lysimeter equipped with a 32 mm diameter PVC monitoring and pump out pipe. There is no storage well nor automatic water level recording mode with this design.

Two lysimeters again 3.2 m x 3.2 m surface area were constructed, one using a sand fill and one using a garbage fill. The design consists of a 36 mil CPE fiber mat reinforced sandwich liner placed on a sand cushion. The lysimeter is approximately 0.4 m deep at the lowest point beneath the clay cover. #2300 woven polypropylene filter


A
A′
32 mm Ø pvc monitor and pumpout location
1m wide polythene sheeting taped to liner material at base
Bentonite seal at surface and at base
Surface replaced to existing grade
Undisturbed Clay Cover
Undisturbed Clay Cover
Sand
Sand
6mm Ø holes wrapped with fiberglass tape
75 mm thick sand cushion
Undisturbed Clay Cover
liner
Plan
3.0 m
32 mm Ø pvc monitor
3.0m
A
A′
Scale 1:100
SCALE 1:20

NOTE : 2 lysimeters to be constructed :
1 to be backfilled with garbage
2 to be backfilled with sand

Surface water run-off collection only at lysimeter 1

Lysimeter backfill to be brought to field capacity before placement of cover

"AS CONSTRUCTED"

Lysimeter Design No. 2

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material was again used to prevent movement of fines from the clay cover into the lysimeter. The thickness of clay cover above the lysimeter was reduced to approximately 0.6 m. Polythene sheeting was again placed around the sides of the excavation prior to backfilling. The clay cover was recompacted in the same manner as before.

Following construction of these two more basic type lysimeters, several operational problems became apparent. The major drawback with this design is the very long time required to remove the water from the lysimeters when they become full. This has been attributed to the slow response time of the lysimeter fill coupled with the narrow diameter of the monitoring pipe. Another drawback to this design is the labour intensive nature of gathering data. As there is no automatic water level recorder, all data must be obtained manually. If measurements of infiltration are required for individual ranfall events, it means that special trips must be made to the site after each event. Obviously, unless personnel from the landfill site can obtain the data, severe economic considerations come into play. Due to the above factors, no reliable data have been obtained from these two lysimeters to date.

RESULTS TO DATE:

Data regarding infiltration at this site have been generated from January 1983 onwards. Continuous water level readings have been obtained at the three sophisticated type lysimeters from mid-March onwards and all three installations appear to be functioning satisfactorily. Monthly records of climate data have been obtained from the weather station located at the nearby Toronto International Airport. Monthly records of total leachate volumes which pass through the collection system have been provided by the Region of Peel. It should be noted that these figures include the sewage from the scale house, site office and maintenance buildings.

A typical response curve for the lysimeter installations is shown on Figure 3. The curve shows a good correlation between a precipitation event and a response in the collection well. A more detailed look at the time of the precipitation event reveals a time lag of between 1 and 6 hours before a response is noted in the well. The other interesting feature is the tendency for a response in the well to continue long after the precipitation event has ceased.

The results which have been collected to the time of writing are presented on Table 1, which follows. These data are also presented graphically on Figure 4. The correlation between the data obtained so far from the three lysimeters is reasonably good, particularly when the results are averaged over the monitoring time period. The plot of average infiltration against time shows that the amount of infiltration, expressed as a percentage of total precipitation, is greatest during

TYPICAL MONITOR WELL RESPONSE CURVE
Lysimeter Installation #2 May 16 - June 15

Fig. 3

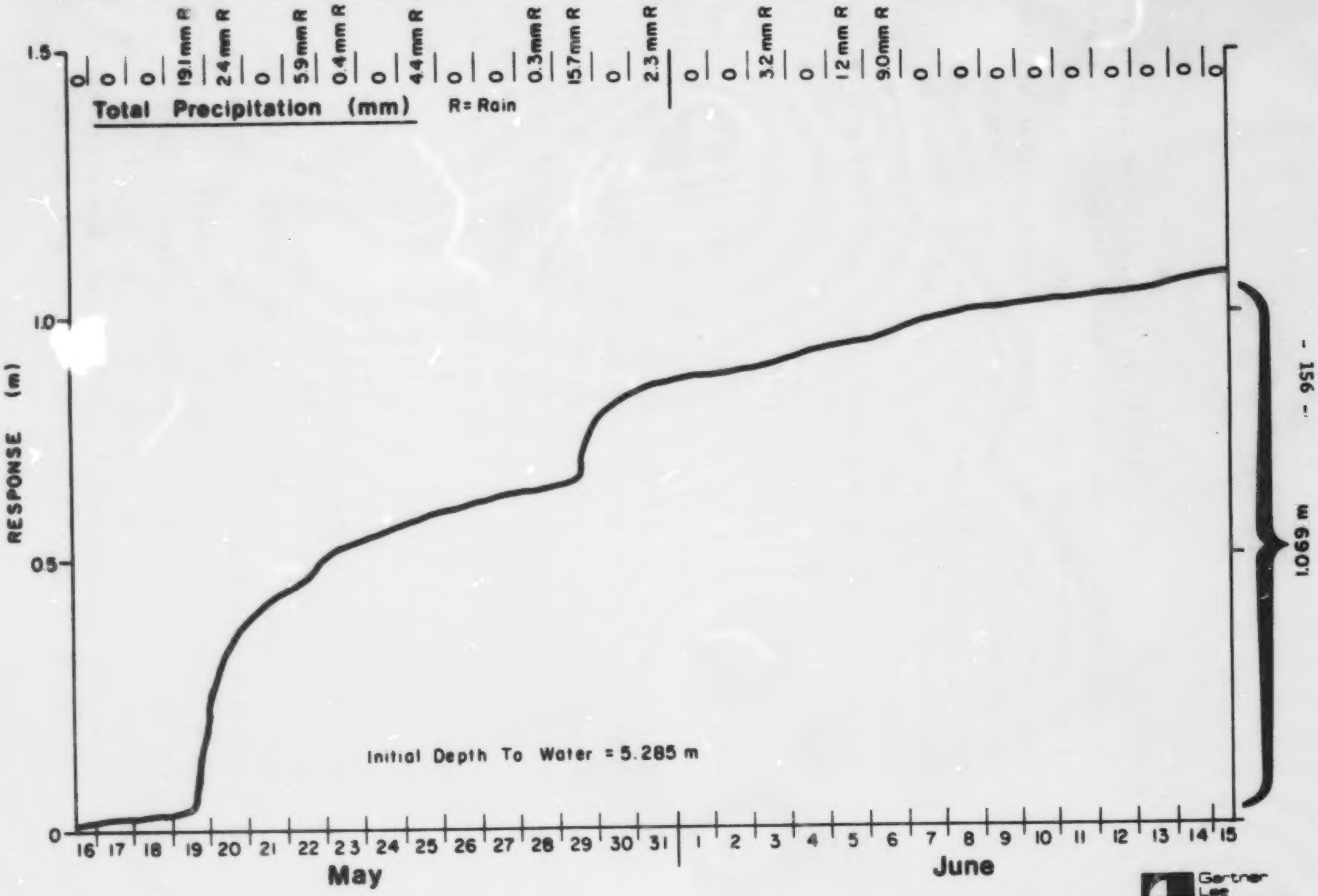

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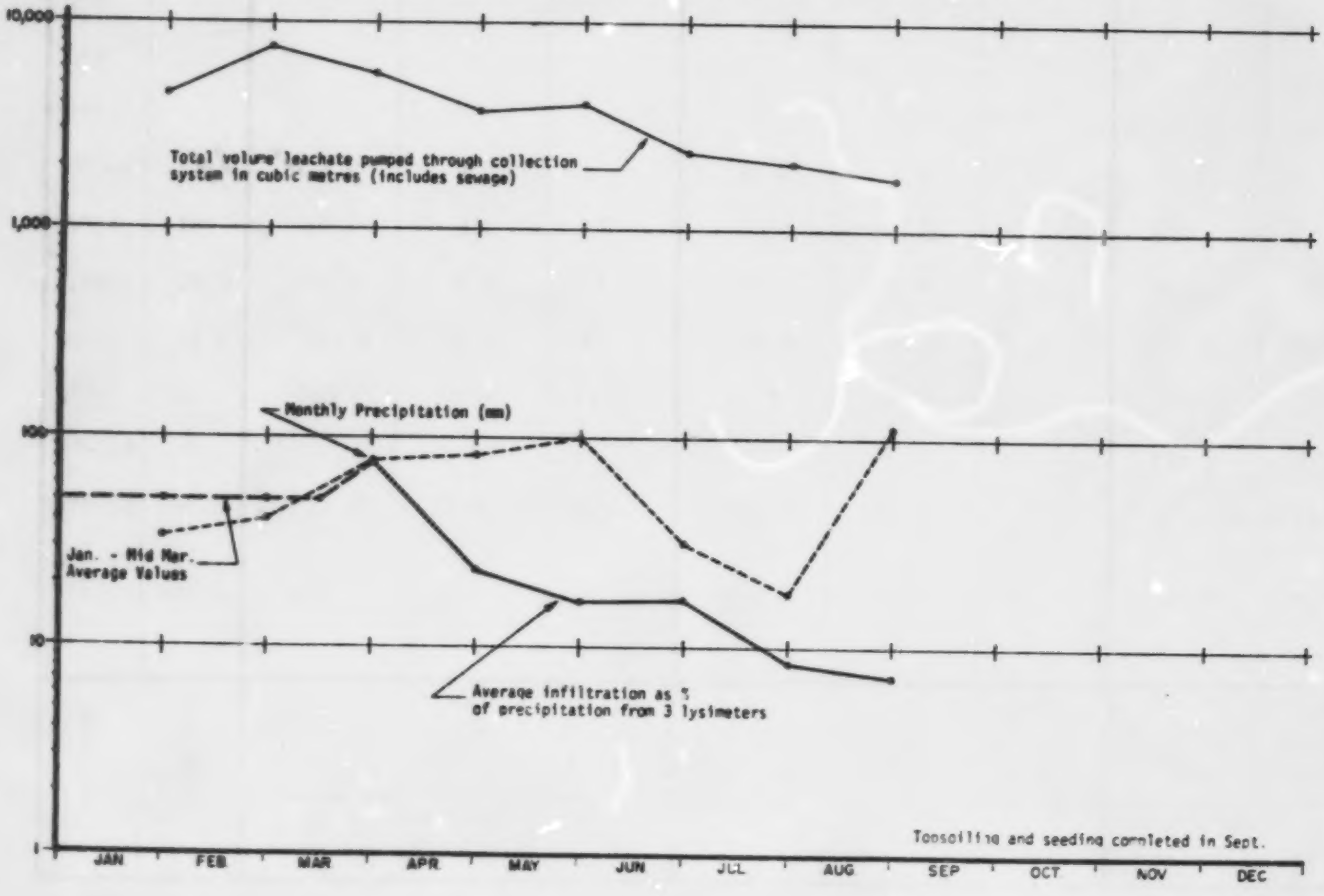
Table 1: VALUES OF LYSIMETER INFILTRATION, MONTHLY PRECIPITATION AND TOTAL LEACHATE VOLUMES

Monitor	Total Precip. (mm Water)	LYSIMETER #1 INFILTRATION			LYSIMETER #2 INFILTRATION			LYSIMETER #3 INFILTRATION			LEACHATE COLLECTION SYSTEM
		VOLUME (LITRES)	EQUIV. DEPTH (mm)	AS % OF PRECIP.	VOLUME (LITRES)	EQUIV. DEPTH (mm)	AS % OF PRECIP.	VOLUME (LITRES)	EQUIV. DEPTH (mm)	AS % OF PRECIP.	TOTAL VOLUME PUMPED
											(cubic m)
Jan. - Mar.17	96	439	43.9	46	407.7	40.8	43	808.5	80.9	63	Jan. 4535
Mar.18 to Mar.31	55.7	513.5	51.4	92	449.5	45.0	81	21st on 157.3	15.7	66	Feb. 7617 Mar. 5756
Apr.	83.2	140.1	14.0	17	86.2	8.6	10	354.3	35.4	43	3723
May	99.5	163.9	16.4	17	208.9	20.9	21	121.6	12.2	12	4035
June	33.0	68.7	6.9	21	63.5	6.4	19	34.4	3.4	10	2377
July	18.3	35.1	3.5	19	3.5	0.35	2	7.3	0.7	4	2090
August	112.2	79.8	8.0	7	34.7	3.5	3	78.1	7.8	7	1750
Sept.											
Oct.											
Nov.											
Dec.											
TOTAL				Average 31%			Average 26%			Average 29	

FIGURE 4

VALUES OF LYSIMETER INFILTRATION, MONTHLY PRECIPITATION AND TOTAL LEACHATE VOLUMES FOR BRITANNIA ROAD LANDFILL SITE



MEASUREMENT OF INFILTRATION
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the spring melt period and decreases through the summer. This agrees well with the plot of total leachate which has been pumped through the collection system. However, the values for total leachate volume are not fully representative of the leachate generated by infiltration within the two completed cells, particularly for the period January to March. During this period, Cell 4 was partially excavated with a portion of the underdrain leachate collector system in place and operational. Consequently, all the precipitation falling within the cell would have entered the collection system. Thus, a significant portion of the so-called leachate would have actually been surface water during this time. Landfilling actually commenced in cell 3 in early March and it has been assumed that much of the precipitation falling within the cell was absorbed by the garbage from April onwards.

The total precipitation between January and August was 497.9 mm of water. The average total amount of infiltration from three lysimeters was 136 mm for the same period. This is equivalent to 27% of the total precipitation . An estimate of the total amount of infiltration which occurred through the covers of Cells 1 and 2, based on adjusted leachate volumes, is between 60-120 mm or 14 to 24% of total precipitation. The variance is due to the amount of surface water which is assumed to enter the leachate collection via Cell 4. Even with the uncertainty associated with the leachate volumes, the correlation between the two methods of measuring infiltration is reasonably good. These initial data suggest that the yearly infiltration at a clay-covered landfill site in southern Ontario may well be in the order of 180 to 200 mm (7 to 8 inches). The equivalent leachate generation rate is 4 Litres per minute per hectare of landfill (0.3 gallons per minute per acre).

Evaluations for 11 full scale landfill sites in West Germany where leachate is collected, indicated that leachate production is dependent on the degree of compaction achieved for the landfill final cover. The results indicated that for landfill sites with a high compaction cover density which was in excess of 0.7 t/m^3, the leachate production rate was within the range of 15-25% of the annual precipitation rate. For those landfill sites classed as having low compaction cover density ie. below 0.7 t/m^3, the leachate production rate was within the range of 25-50% of the annual precipitation rate. The in-situ density at the Britannia Road site was about 2 t/m^3 which would place it within the high compaction category. Since our results to date indicate an infiltration equivalent to between 14 to 27% of precipitation at this site, these data correlate well with the results from West Germany.

TASKS REMAINING:

Two tasks remain outstanding at this site, during this first phase of the study. A calibration exercise has to be completed at the lysimeter installations. A known amount of water is to be slowly applied to the ground surface such that it all infiltrates into the lysimeter and the response measured at the well. In this manner, an estimate of the efficiency of the lysimeters can be obtained.

Additional monitoring of the lysimeters is planned through the forthcoming winter and snowmelt period in order to provide a full 12 months monitoring data.

PHASE 2 OF THE STUDY:

The lysimeter design arising from Phase 1 appears to function satisfactorily such that a similar design can be adopted during Phase 2 of the project. The second phase is intended to measure infiltration through various types of landfill cover materials, located in various physical settings such as geographic location, slope vegetation etc. From here, it should then be possible to compare the actual amount of infiltration with that predicted from the empirical values and drainage equations presently used in landfill cover design.

CONCLUSIONS:

A lysimeter installation to measure the actual amount of infiltration through a landfill cover has been developed and field-tested. Preliminary results indicate that infiltration through a well-compacted clay cover material may amount to about 25% of the total annual precipitation. Although further verification of these data are necessary, the implication is that the yearly infiltration through a clay cover at a landfill site in southern Ontario is likely to be in the order of 180 to 200 mm (7 to 8 inches). The equivalent leachate generation rate is about 4 Litres per minute per hectare (0.3 gallons per minute per acre). With slight modifications this type of lysimeter can be used to assess infiltration through landfill covers with various scenarios of slope, type of material, degree of compaction, vegetative cover and climate types.

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THE USE OF MARSHLANDS IN WASTEWATER TREATMENT

- ONTARIO'S RESEARCH PROGRAMS -

BY

S. A. BLACK

OVERVIEW OF A PAPER FOR PRESENTATION
AT THE
TECHNOLOGY TRANSFER CONFERENCE # 4
RESEARCH ADVISORY COMMITTEE
ONTARIO MINISTRY OF THE ENVIRONMENT
NOVEMBER 29-30, 1983

Abstract

The Use of Marshlands In Wastewater Treatment

S. A. Black
Ministry of the Environment

The use of aquaculture concepts for wastewater treatment is receiving increased attention. Systems studied to date, have included both natural and artificial marshlands, ponds, raceways and other structures based upon various combinations of rooted and free-floating vegetation. The use of both artificial and natural marshlands for the treatment of municipal wastewater is being actively evaluated in Ontario.

This paper defines the current status of marshland treatment technologies and evaluates their potential for routine use in municipal wastewater treatment. In so doing, the paper presents an update of the ongoing research studies at the Listowel artificial marsh facility and outlines details of the Bradford natural marsh study, the Cobalt Artificial Marsh Study and the Port Perry marshland treatment demonstration project.

INTRODUCTION

Use of marshes for year-round treatment of sewage has been under study by the MOE since 1980. Although research facilities include a small scale natural marsh at Bradford, efforts have been primarily focused on the use of artificial systems. A small, multi-celled, artificial marsh at Listowel has provided the basis for development of preliminary design and operating criteria. A more recently constructed experimental marsh facility at Cobalt will provide essential information for implementation of this technology in Northern Ontario. A full-sized demonstration facility is under construction at Port Perry which will process the entire community's wastewater flow of 0.4 MGD.

This paper describes Ontario's ongoing research activities, defines the current status of marshland treatment technology and addresses the potential application of this alternative for wastewater treatment.

1 PROGRAM OBJECTIVES

The primary objectives of the Marshland Study Program are:

a) to determine the capacity of natural and artificial marshes to renovate wastewater on a year-round basis in Northern and Southern Ontario and,

b) to define precise guidelines for the design and operation of marshland wastewater treatment systems.

Several secondary objectives relate to the fundamental understanding of marsh treatment systems and to the development of non-routine operational procedures. These include:

c) an assessment of the role of various storage compartments (soil, plants, litter, micoorganisms) in the removal and ultimate fate of wastewater constituents;

d) an investigation of nuisance organisms generated within the marsh environment, their significance and possible mitigative measures;

e) an assessment of the long-term impacts of wastewater additions on wetlands;

f) an evaluation of the impact of vegetation harvesting on effluent quality and litter accumulation and an assessment of potential uses for the harvested biomass.

2 DESCRIPTION OF STUDY SITES

The following research facilities are currently under study as part of the marshland program:

a) Listowel - An artificial marsh comprised of 5 independent systems has been constructed at Listowel. Three system receive pretreated wastes from an existing lagoon and 2 systems receive effluent from a completely mixed pretreatment aeration cell. This facility is unique in the world because of the flexibility in operating conditions including hydraulic loading, depth and retention times. It also includes two different geometric configurations, namely, serpentine or channelled marshes and open rectangular marshes. Construction was completed in July and sewage additions commenced in August of 1980. Funding for the construction of this facility was provided by the Provincial Lottery.

b) Bradford - This 1400 m^2 natural marsh was physically isolated from interactions with adjacent marsh areas. The facility is fully instrumented to allow detailed measurements of water budgets. Background nutrient fluxes were monitored for 2 years and sewage additions (blend of raw sewage and final effluent from the Bradford STP) commenced in June, 1981.

c) Cobalt - Construction of two small artificial marshes in Northern Ontario was funded by the Ministry of Norther Affairs. One plot was located in a mine tailings basin to establish the potential for conversion of the entire basin to a full-scale marsh wastewater treatment facility for this community. The second plot was built in more representative Northern Ontario soil. Final construction and planting with cattails was completed in July, 1982.

d) Other Sites - Although research studies at the above mentioned study sites will define the wastewater renovation capabilities of marshes, the short study span will not provide information on their long-term capacity (i.e. 10-20 years) to accept wastewaters. Consequently, studies of two wetland sites (Regina Mundi College, Southwestern Region and Sherwood Farms in West-Central Region) which have received pretreated wastewaters over extended periods of time were initiated in 1982.

3 TREATMENT CAPABILITIES

Several research studies throughout North America have demonstrated the viability of marshland treatment of wastewater but to date no design and operating crtieria for routine application of this technology have been developed. Wastewater renovation capabilities documented in scientific literature are highly variable and reflect the different hydraulic loading rates, wastewater characteristics and climatic conditions. Based on a recent worldwide literature review, Reed and Bastian (1980) provide the following broad ranges of removal efficiencies for major wastewater constituents:

Constituent	Range of Removal Efficiency (%)
Suspended Solids (SS)	60 - 90
Biological Oxygen Demand (BOD_5)	50 - 96
Total Nitrogen (TN)	30 - 98
Total Phosphorus (P)	10 - 90

Clearly, further effort is required to define the wastewater renovation capabilities of wetlands and develop reliable engineering criteria.

Although U.S. research efforts precede Ontario's program their studies have focused on the summer application of dilute wastewaters, to natural wetlands. Ontario studies emphasize year-round application of more concentrated wastewaters, to minimize pretreatment costs and eliminate the need for winter storage. Since little effort has been directed, elsewhere, to the use of artificially created marshes, studies at Listowel have generated world-wide interest. A brief synopsis of the results obtained to date at each of the Ontario study sites is provided below:

a) Listowel - The first year of operation at the artificial marsh site in Listowel produced highly variable results. Effluent quality during the first three months superseded the capabilities of even the most advanced conventional processes. A dramatic decline in treatment efficiency during the subsequent winter months coincided with a three fold increase in hydraulic loading rates. Variable results were achieved during the following spring and summer season, despite reduced hydraulic loads.

Clearly, the data generated during the first 12 months period reflect the instability of the newly created marshes, the large initial absorption capacity and the variable hydraulic loadings. Nevertheless, the first year of operation provided valuable insight into the numerous factors which impact on marsh performance and led to effective adjustments in the mode of operation. Throughout this period, it was also apparent that, despite identical operating conditions, the channelled marshes consistently outperformed the open marsh systems. Short-circuiting of wastewaters was identified as the primary cause and design criteria were evolved to address this problem.

The consistent effluent quality achieved during the second year of operation was indicative of a more stable ecosystem. Figures 1 to 5 illustrate the wastewater renovation capabilities of a channelled marsh system (System IV) for this period. The results are presented both in terms of actual concentrations (mg/l) of major constituents in the influent and marsh effluent and in terms of mass balances (g/m^2). The latter values are more representative of marsh performance since the concentrating and diluting effects of evapotranspiration and precipitation are taken into account. This is particularly relevant during prolonged periods of hot, dry weather when evapotranspiration significantly reduces the volume of outflow from the marshes, and thus concentrates the wastewater constituents.

Consequently, high reductions in both BOD and SS were achieved on a year-round basis (Figures 1 and 2). Minor declines in removal efficiencies during ice-free periods reflect the generation and decomposition of marsh biota, particularly phytoplankton. Concentrations of both constituents in the marsh effluent were well below the current 15 mg/l design guidelines for conventional activated sludge secondary plants.

High removal rates for total phosphorus were also sustained throughout the year (Figure 3) and concentrations of this element remained below the 1.0 mg/l objective for conventional processes with phosphorus removal. Maximum phosphorus concentrations in the marsh effluents coincided with the summer period and reflect both the increase in net loading and the concentrating effects of evapotranspiration. With continuous P removal in the pretreatment step, P concentrations of <0.3 mg/l should be achieved. Nitrogen removal rates were far more variable with high removal efficiencies evident only during the summer season (Figures 4 and 5). Elevated ammonia concentrations during the winter months could limit the application of this technology in areas with very restrictive receiving waters. However, a more suitable pretreatment option could eliminate this difficulty.

b) Bradford - Hydraulic loading rates and concentrations of major constituents in the wastewater influent closely parallel the Listowel operation. Mass balance of data, spanning a 7 month period subsequent to sewage additions, show reductions in BOD, SS and TN comparable to the artificial marshes at Listowel. A 60% reduction in P loading is, however, significantly lower than the >80% removal achieved at Listowel. Since the marsh site at Bradford was already enriched with phosphorus prior to sewage addition, its reduced ability to assimilate large quantities of this element is not unexpected.

c) Cobalt - Continuing problems with severe exfiltration from the mine tailings plot (#2) have precluded the establishment of a routine water quality monitoring program. Efforts are still underway to correct the problem. While some information has been generated from the other plot (#1), the data span less than 6 months and are thus extremely preliminary.

To date, reductions in SS, BOD, TN and P compare favourably with Listowel results, with effluent quality meeting secondary treatment objectives. The raw sewage influent is quite dilute as a result of the deteriorating condition of the community's sewer and water mains. Consequently, higher hydraulic loading rates are being applied at this site.

d) Other Sites - A preliminary survey of Ontario wetlands which have received wastewater effluents over an extended period of time (>10 years), revealed only two sites which were considered suitable for further study.

Wastewaters from an extended aeration plant at the Sherwood Farm Poultry Processing Operation have been discharged to a 1 ha wetland since 1972. Prior to this date, the wastes were piped to a lagoon which subsequently discharged into the wetland. Historical air photos show a progressive change from a wet meadow ecosystem to a cattail dominated marsh, following addition of wastewaters. Based on company production records, current effluent loadings of 178 m^3/day to the marsh appear to be representative of the past 15 years. Concentrations of wastewater constituents in the effluent discharged to the wetland are quite variable (5.8 to 10.3 mg/l P; 0.3 to 5.5 mg/l NH_3; 3.9 to 28.7 mg/l BOD). Historical data on effluent quality are extremely sporadic. Wastewaters are retained in the marsh for only brief intervals, suggesting extensive short-circuiting. Despite the brief retention time, and the many years of wastewaters additions, all major wastewater constituents are reduced by some 40% during passage through the marsh.

A second study site, adjacent to Regina Mundi College in London, receives effluent from a contact stabilization plant, built in 1963. The entire marsh/pond covers an area of 8 ha. The small wastewater loading of 50 m^3/day was found to be insignificant when compared to other sources of input such as runoff from the watershed area. Consequently, information gathered from this site is of little use in assessing long-term impacts of wastewaters on wetlands.

e) Generation of Nuisance Organisms - Mosquitos and midges are the primary organisms of concern in marshland treatment of wastewaters. Studies at Listowel revealed that the numbers of midges generated from the marshes were small in comparison to the production of these organisms in conventional lagoons. On the other hand, vast numbers of mosquitos were produced in the marsh environment, reaching peak populations of 9,000 larvae per m^2. Four species of mosquitos were identified, however, none are significant man-feeders. The man-feeding *Mansonia perturbans* which typically occurs in wetlands was not found at the Listowel site. Adult trapping data showed that mosquitos produced in the Listowel marshes were not contributing to increases in populations beyond a 1 km radius. The only mosquito specie of concern is *Culex pipiens*, a probable vector of St. Louis Encephalitis. Consequently, chemical control would only be required in the event of Encephalitis activity. A recently registered bacterial insectide, *Bacillus thuringiensis* which provided 90% control in trials at Listowel, is recommended for this purpose.

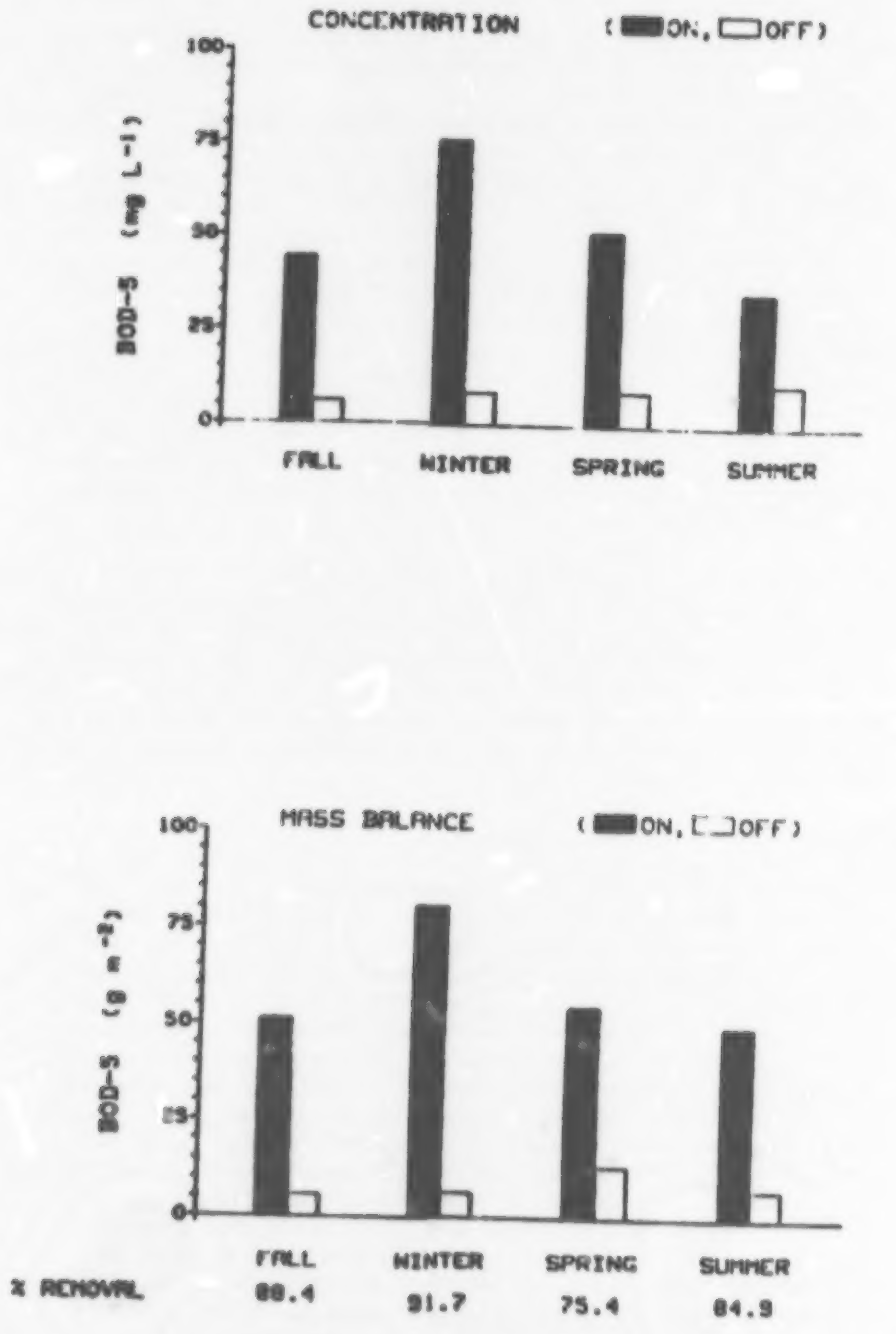

FIGURE 1: REDUCTION IN BOD-5 IN SYSTEM 4, 1981-1982.

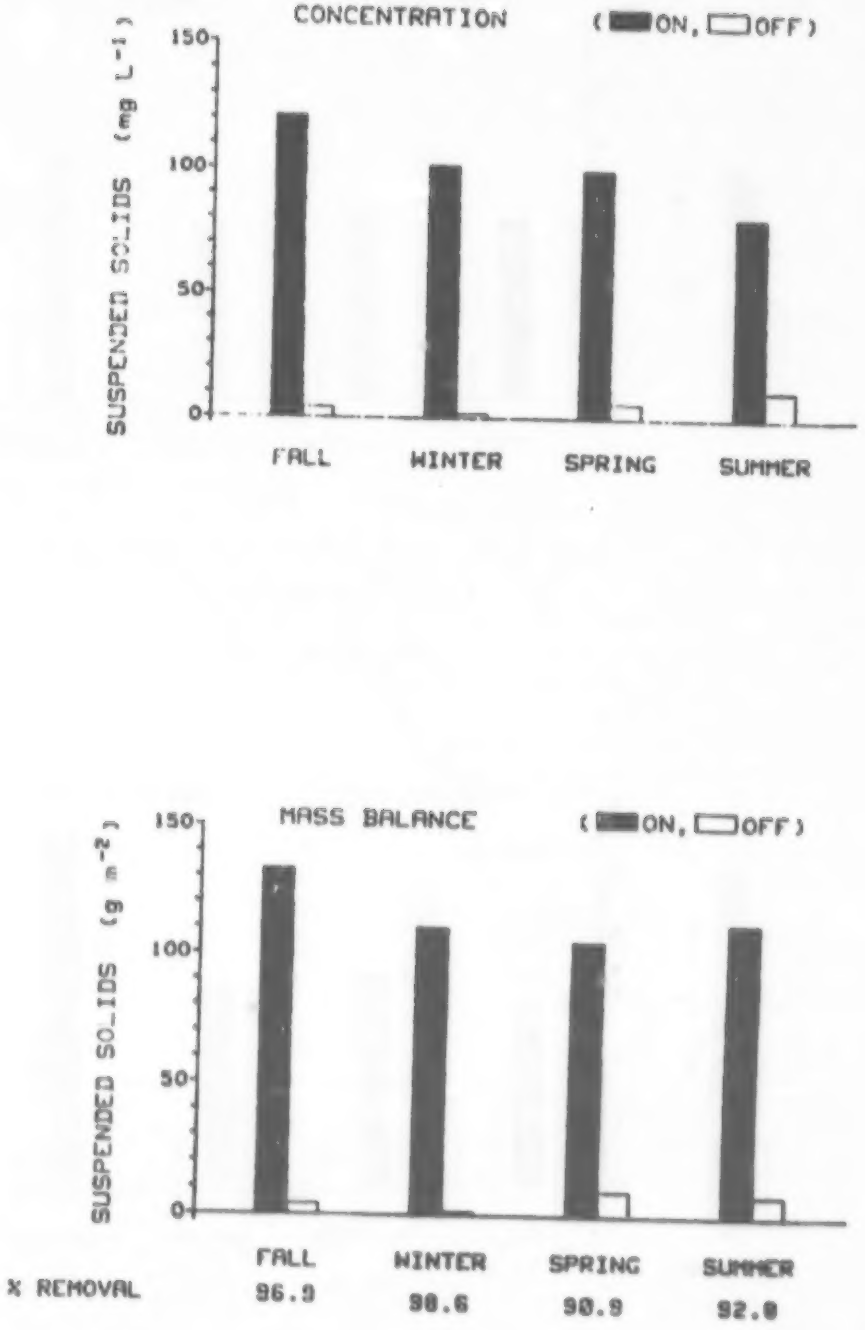

FIGURE 2: REDUCTION IN SUSPENDED SOLIDS IN SYSTEM 4, 1981-1982.

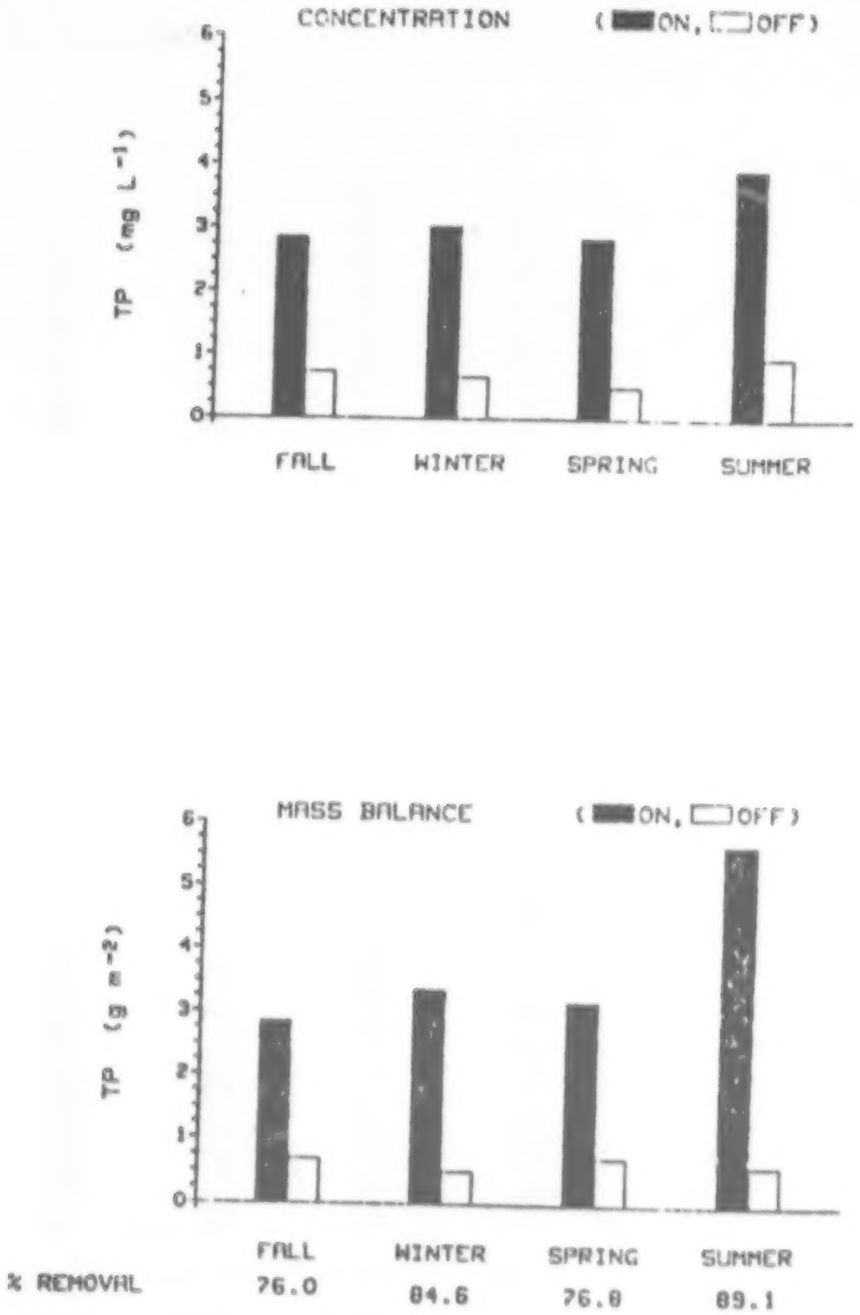

FIGURE 3: REDUCTION IN TOTAL PHOSPHORUS IN SYSTEM 4, 1981-1982.

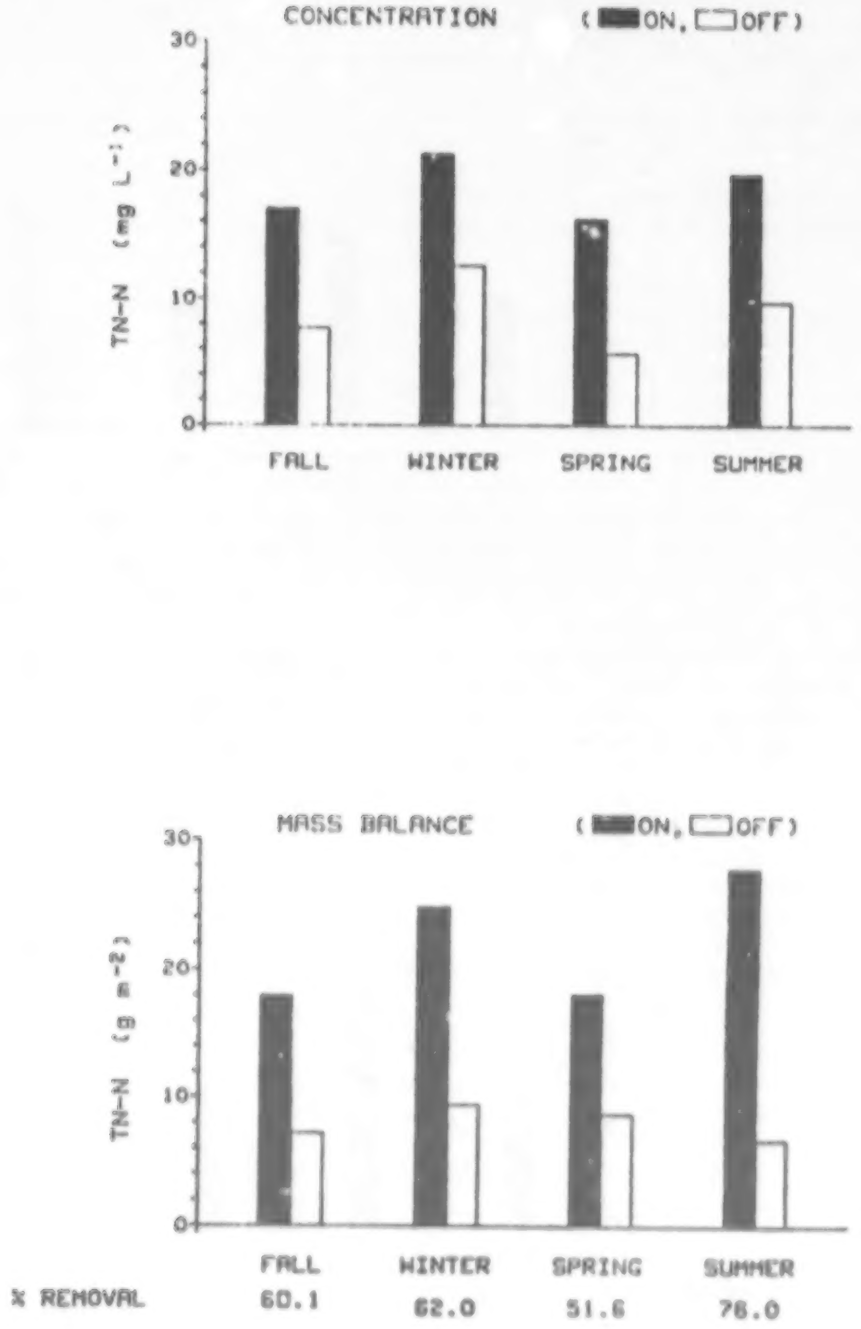

FIGURE 4: REDUCTION IN TOTAL NITROGEN IN SYSTEM 4, 1981-1982.

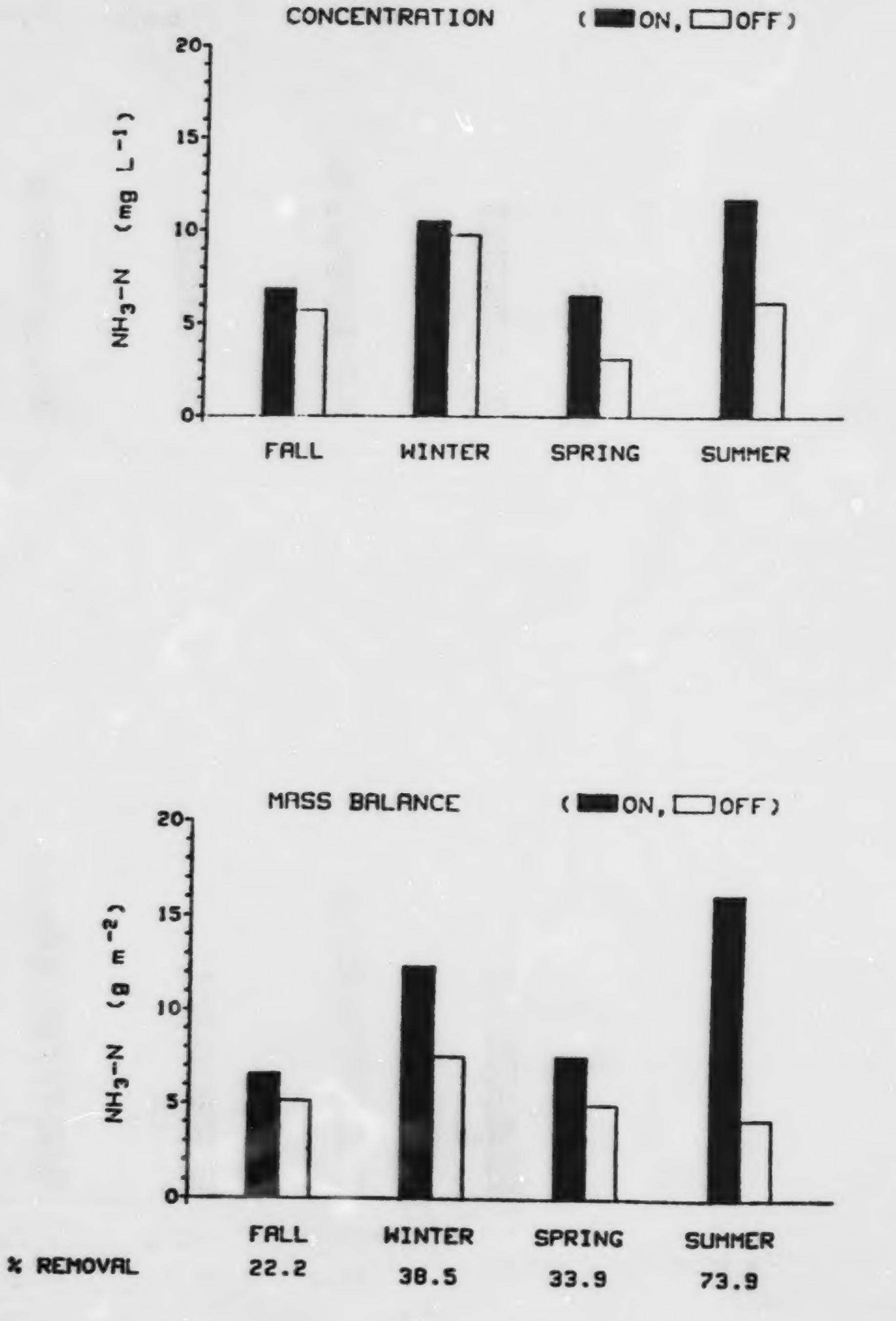

FIGURE 5: REDUCTION IN AMMONIA IN SYSTEM 4, 1981-1982.

4 PRELIMINARY DESIGN CRITERIA

Based on studies at Listowel, the following preliminary design criteria have been developed for artificial marshland treatment systems. Within the limits of site specific characteristics, these criteria could also be used as guidelines for development of natural marshes for wastewater renovation purposes.

a) Site Selection - Artificial wetlands can be constructed almost anywhere. In Ontario, experimental systems have been built in heavy clay soils, organic soils and in an abandoned mine tailings basin. Since grading and excavating represent a major cost factor, topography is an important criterion in the selection of an appropriate site.

b) Vegetation - Cattails, Typha. spp. are cosmopolitan in distribution, hardly capable of thriving under diverse environmental conditions, easy to propogate and thus represent an ideal plant species for artificial marshes. They are also capable of producing a large annual biomass and provide greater potential for N and P removal when harvesting is practised. Cattail rhizomes planted at approximately one meter intervals will produce a dense stand within three months in Southern and Central Ontario. Planting at reduced densities or merely allowing for natural invasion of vegetation will achieve the same objective but over a more extended period of time.

c) PreTreatment of Raw Sewage - To reduce costs, minimal pretreatment of wastewater prior to discharge to a marsh is desirable. The level of pretreatment will, however, influence the quality of the final marsh effluent and therefore effluent objectives must be taken into consideration.

The two pretreatment options at Listowel are now regarded as inadequate. The conventional lagoon represents inefficient use of land and contains elevated hydrogen sulphide concentrations in winter. The complete mix aeration cell is energy intensive and allows for the carryover of high levels of volatile solids, leading to sludge accumulations and oxygen stress in the marshes.

It is anticipated that a 5-10 day retention facultative aeration cell may represent the ideal pretreatment alternative. This option should adequately reduce BOD and SS concentrations in the raw sewage prior to discharge to a marsh treatment system, minimize energy costs and provide for efficient use of land resources.

Although phosphorus removal in the Listowel marshes is substantial (up to 98%), on a long-term basis, efficiencies are expected to decline since no permanent escape mechanisms, such as denitrification exists for this element. Therefore, phosphorus reduction by chemical addition is recommended in the pretreatment step.

d) Marsh Configuration - Studies at Listowel have demonstrated the significance of the geometric configuration of an artificial marsh to performance efficiency. In large open marshes, short-circuiting of wastewater will occur, leading to reduced treatment efficiency. Consequently, marsh systems should be designed with length to width ratios equal to or greater than 10:1 or alternatively with internal baffling to ensure proper flow distribution.

e) Hydraulic Loading - Results obtained at Listowel suggest that hydraulic loading rates of 200 m^3/ha/day of pretreated wastewater will produce a high quality effluent. Further testing is required to assess marsh performance at higher hydraulic loadings.

f) Hydraulic Detention Time - For optimal performance, a 5 to 10 day detention period for wastewater within the marsh is required. At a constant hydraulic loading, detention time will be influenced by two major factors, namely, evapotransportation in summer and ice formation in winter. High evapotranspiration rates will increase detention times and cause stagnation and severe anoxia, whereas ice formation will decrease detention time. Maintaining low summertime liquid depths (<10 cm) and raising water levels in the marsh (>30 cm) prior to the onset of winter conditions will remedy these problems. Consequently, artificial marshes must be designed to provide flexibility in the regulation of liquid depths.

g) Land Requirements - At a loading rate of 200 m^3/ha/day, a 1 MGD (4,546 m3/day loading of wastewater would require some 60 acres (24 ha) of marsh and additional land for internal flow regulation and pretreatment facilities. This compares favourably with conventional seasonal discharge lagoons which require some 100 acres (40 ha) for an equivalent sewage loading.

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THE HAMILTON STUDY: RELATIONSHIP BETWEEN OUTDOOR AND INDOOR AIR QUALITY IN HOMES AND ELEMENTARY SCHOOLS

L.D. Pengelly*, W. Furlong, C. Goldsmith, and S. Toplack

Dept. of Medicine, McMaster University

Measurements have been made of indoor and outdoor levels of nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and suspended particulate material (SPM) using a multipollutant sampler which we have developed (1). Observations were made for two 5-day sessions at each site, once during the heating season (H) and once during the non-heating season (NH). Paired indoor and outdoor observations were made during the same 24-hour sampling period at each site using the same instrumentation. Complete sets of paired data were obtained for 16 schools in both seasons, for 20 homes in the non-heating season and 14 of that 20 in the heating season. Data were much more difficult to obtain from homes than schools. The study showed patterns of results which were systematic for each pollutant, but differed between pollutants. Most of the results reported here will be in terms of indoor-outdoor ratio (I/O) expressed as percent, in the non-heating or heating season. For NO_2, in schools the I/O ratio was close to 75%, and was found to be independent of heating season, with indoor levels strongly correlated with outdoor levels. In homes, the I/O ratio depends on the presence of indoor sources, such as smokers, or gas stoves. In the absence of these, there is relatively little effect of heating season, and the ratio is about 80%. Smokers were not found to have an effect on indoor NO_2 levels in either season, but gas stoves were associated with markedly increased ($P < 0.001$) levels of NO_2 in both seasons, contributing an additional 13 ppb (NH) and 25 ppb (H). This resulted in homes with gas stoves having an I/O ratio of 1.8 (NH), 2.1 (H).

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* The final report is presently under review. For more information, please contact the author.

For SO_2, in schools, the I/O ratio was about 22%, independent of heating season, indoor levels being correlated with outdoor levels ($P < 0.01$). In homes, the indoor level depends on heating season, and is poorly correlated ($P > 0.05$) with outdoor levels in either season. The I/O ratio = 64% (NH), 15% (H) (SE 13%). For SPM, in schools, I/O ratio was 90% (NH), 140% (H), but there was no significant correlation with outdoor levels or season. In homes, I/O ratio was 134% (NH), 101% (H). In the non-heating season there was no significant correlation with outdoor levels, or the presence of smokers. In the heating season, there were significant effects from outdoor levels and smokers. The presence of smokers added 35 $\mu g/m^3$ of particulate to indoor levels.

The results of this study show that in schools, both for NO_2 and SO_2, indoor levels are well correlated with outdoor levels, independent of heating season, whereas indoor particulate levels are variable, frequently higher than outdoor levels, and uncorrelated with outdoor levels or heating season.

In homes without indoor sources for NO_2, there is little effect of heating season, and the indoor levels are correlated with outdoor levels. Gas stoves are a major indoor source, giving rise to indoor concentrations double the outdoor concentrations, during both heating and non-heating seasons. Levels of SO_2 are low indoors, poorly correlated with outdoor levels, and strongly dependent on heating season, being much lower during the heating season. Suspended particulate material levels indoors were generally higher than outdoors, and poorly correlated with outdoor levels in either season. During the heating season, however, the presence of smokers contributed substantially ($P < 0.01$) to the particulate load, but had no effect on the NO_2 or SO_2 levels.

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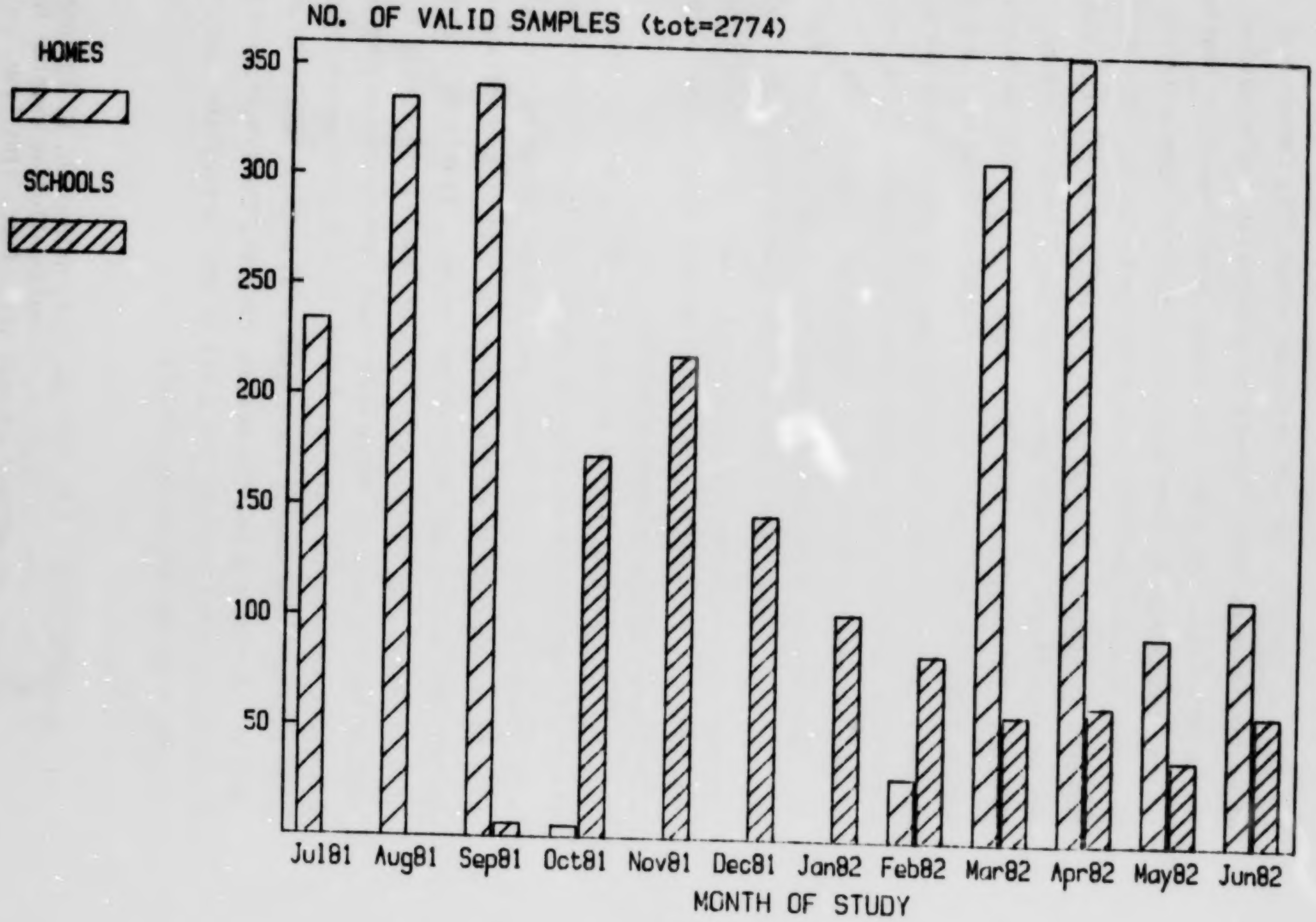
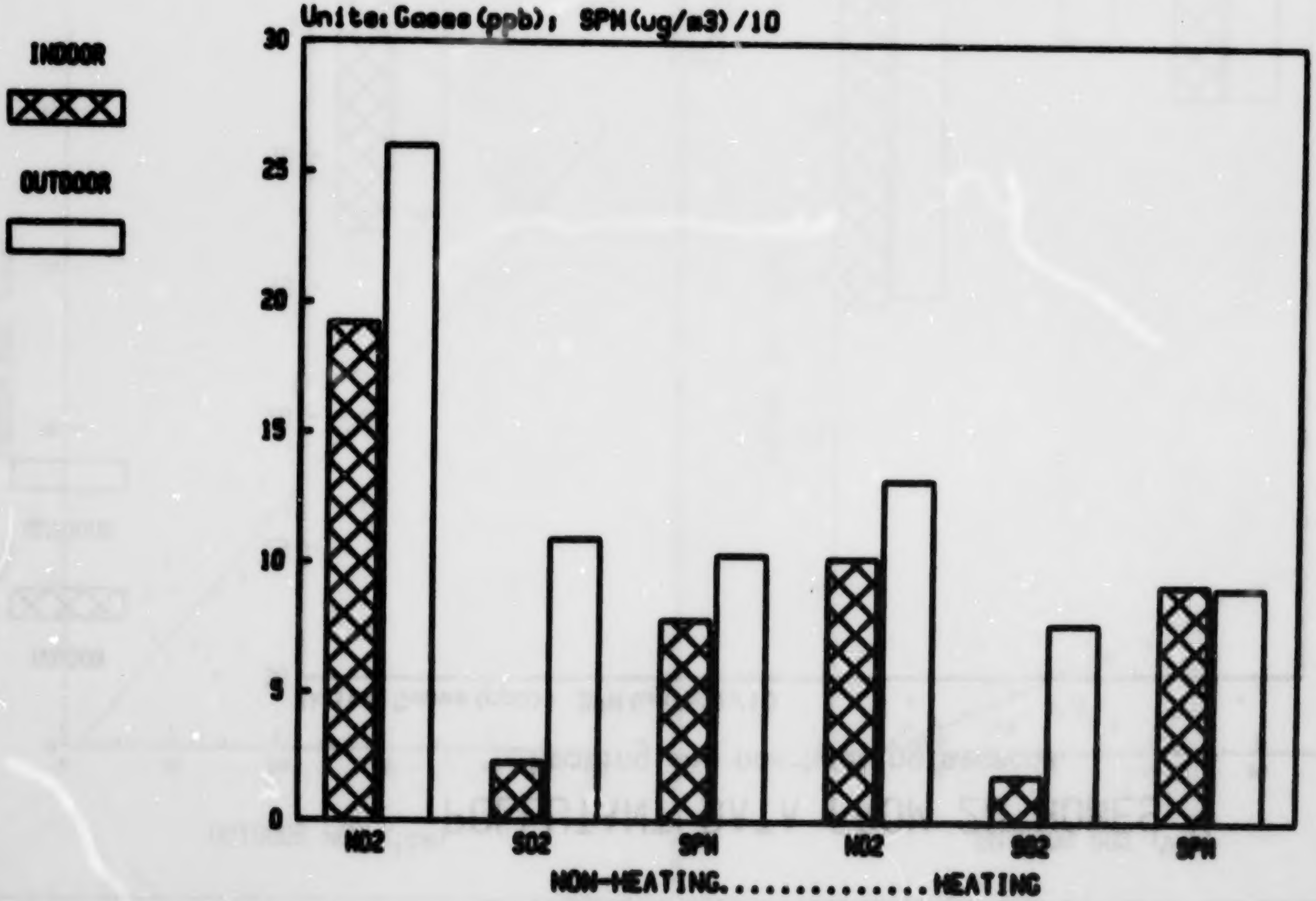
I/O STUDY: VALID SAMPLES

For both homes and schools.

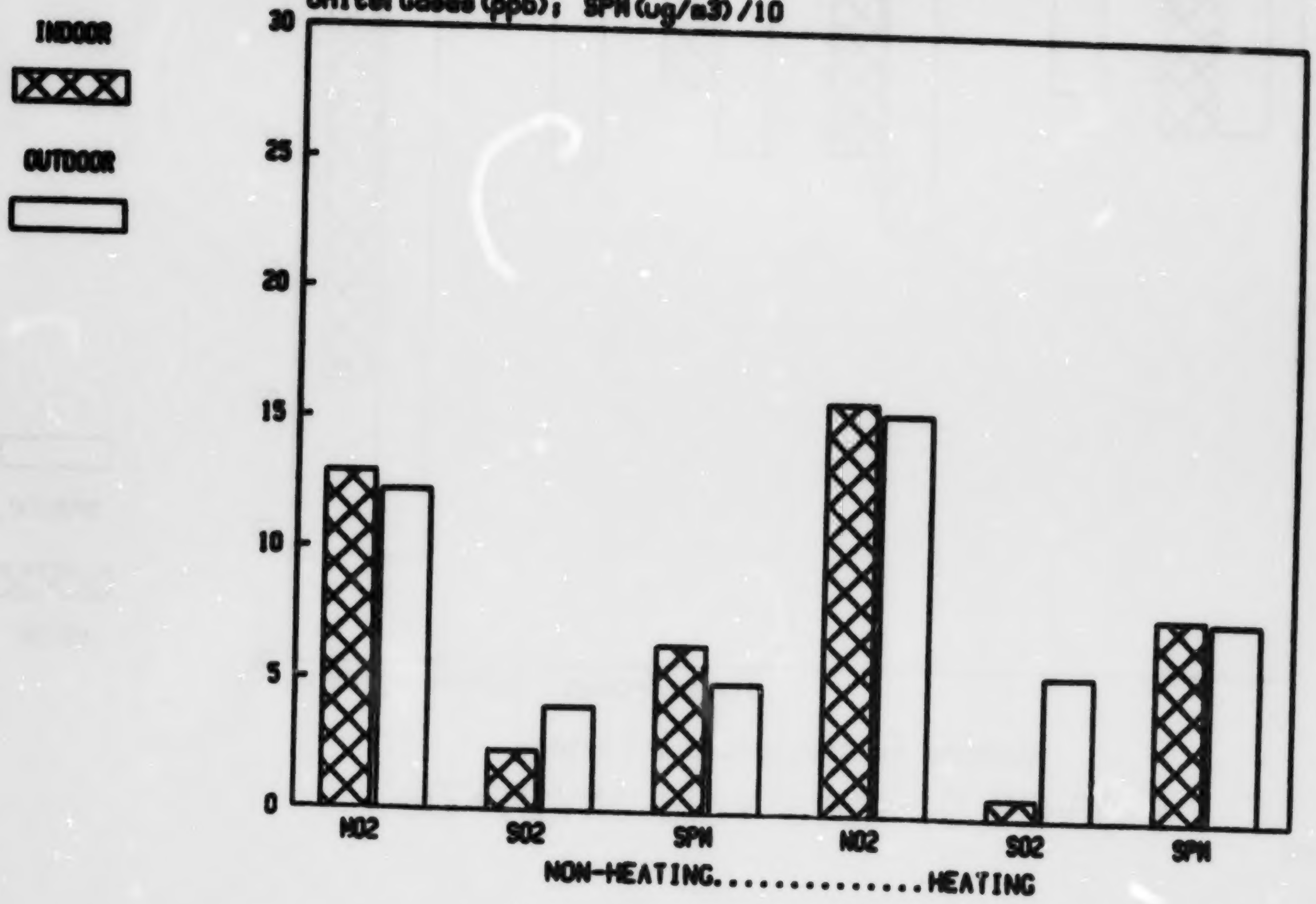


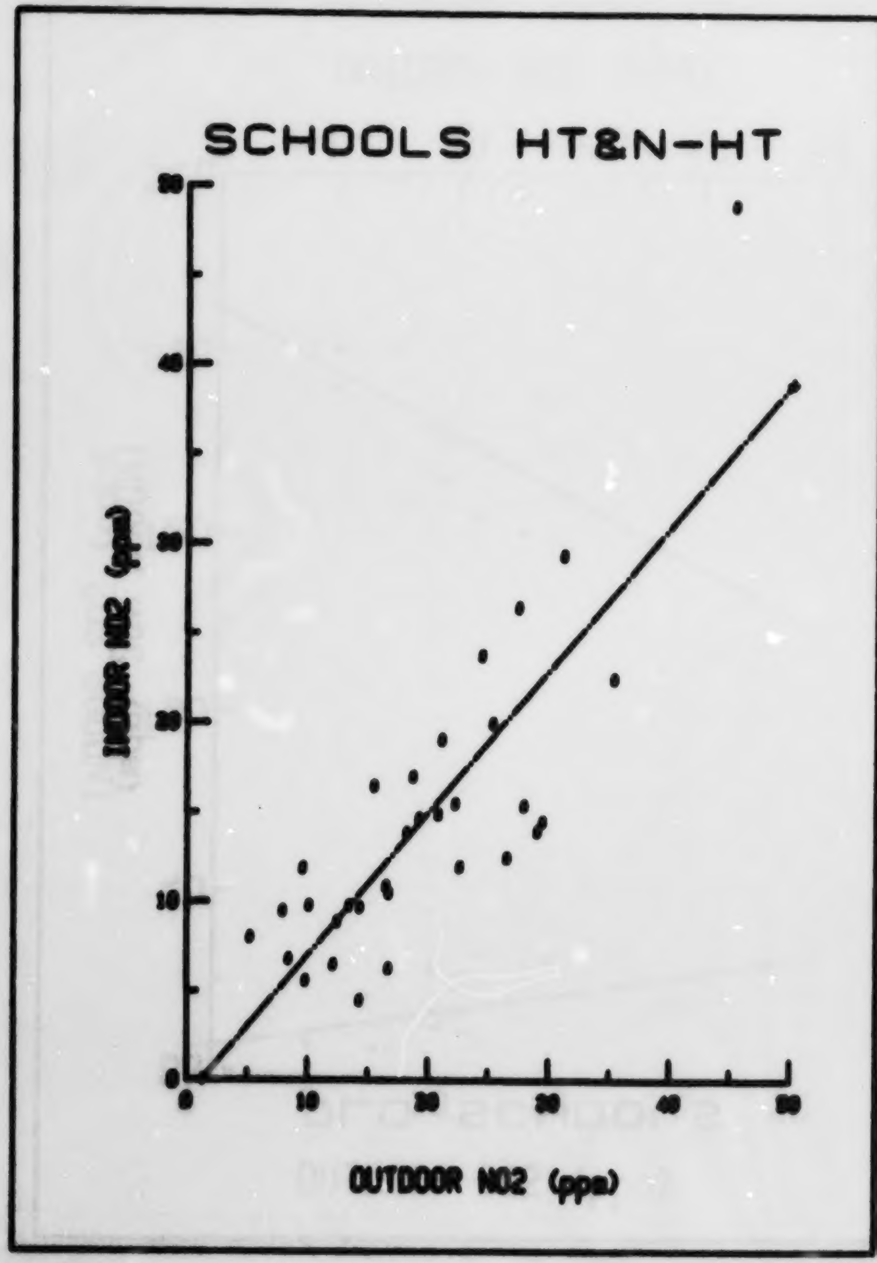


POLLUTANT DATA FROM 16 SCHOOLS
heating and non-heating seasons.

POLLUTANT DATA FROM 20 HOMES

heating and non-heating seasons.




SCHOOLS HT&N-HT
INDOOR NO2 (ppb)
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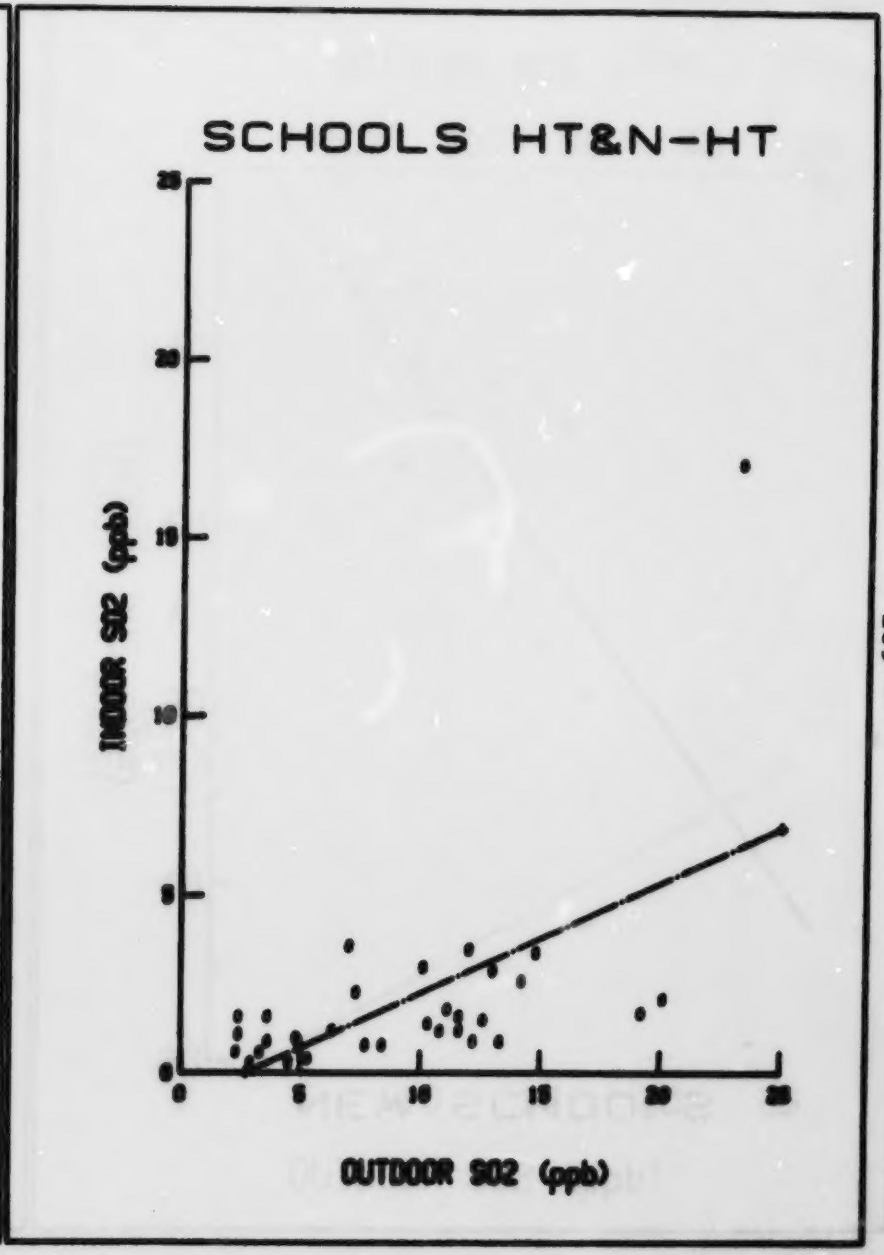
40
50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OUTDOOR SO2 (ppb)
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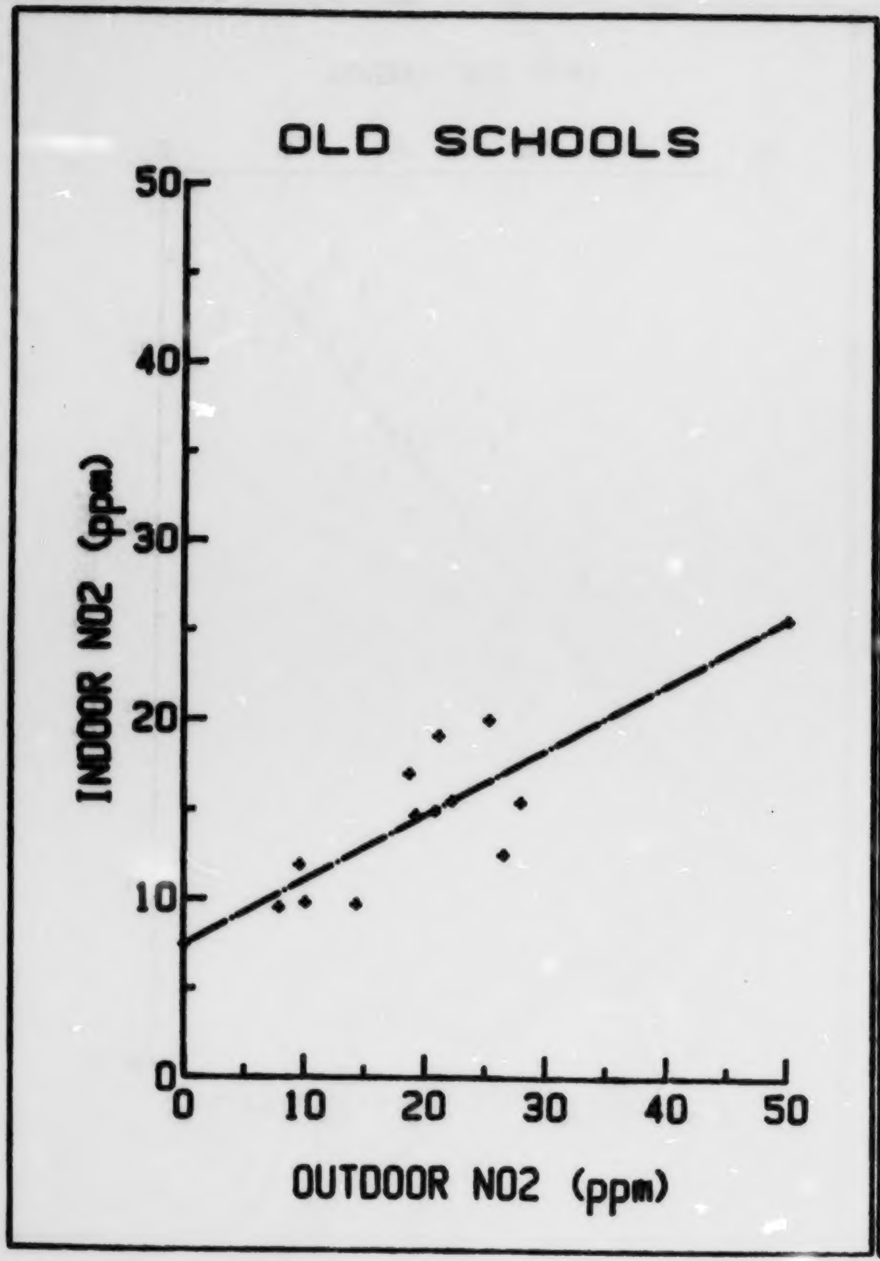
15
20
25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OUTDOOR NO2 (ppm)
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30
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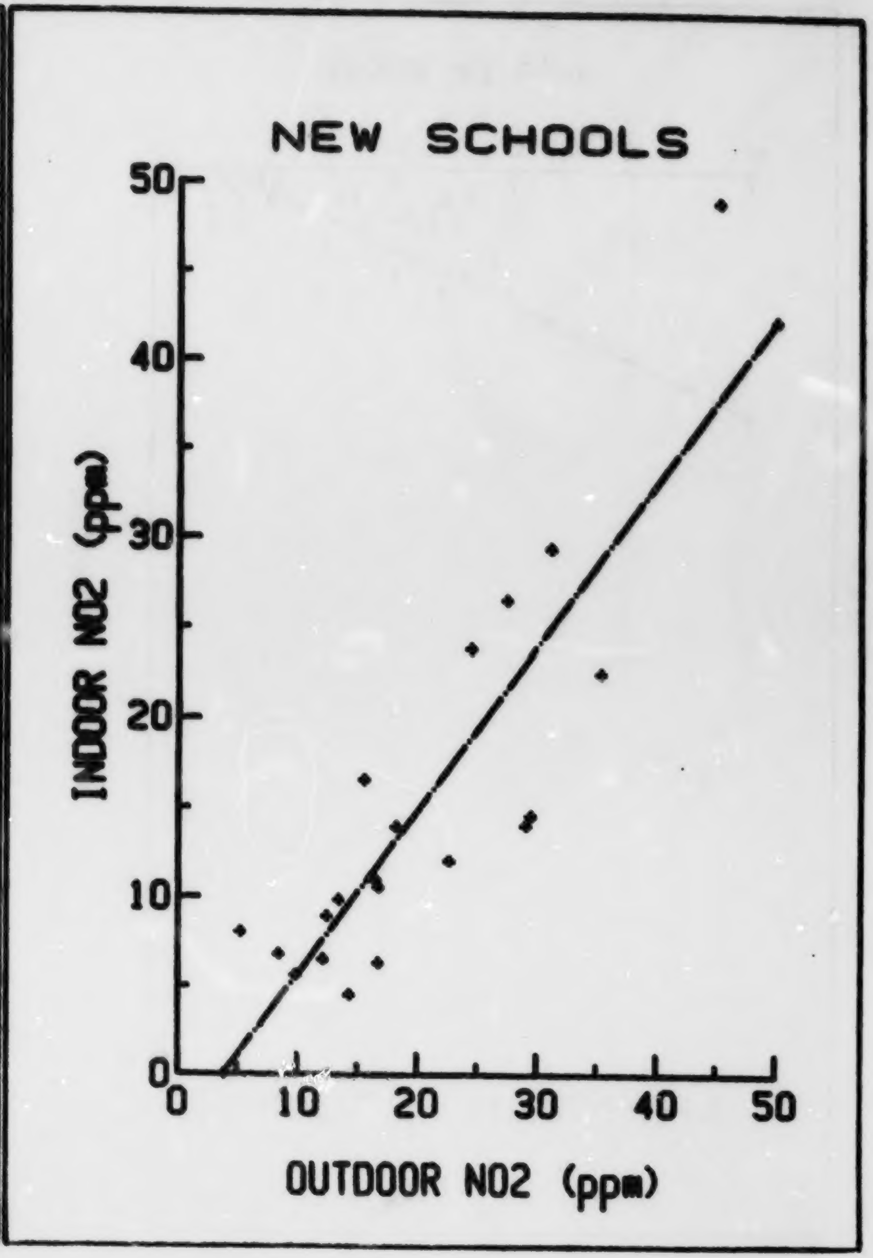
50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10
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30
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OUTDOOR NO2 (ppm)
0
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40
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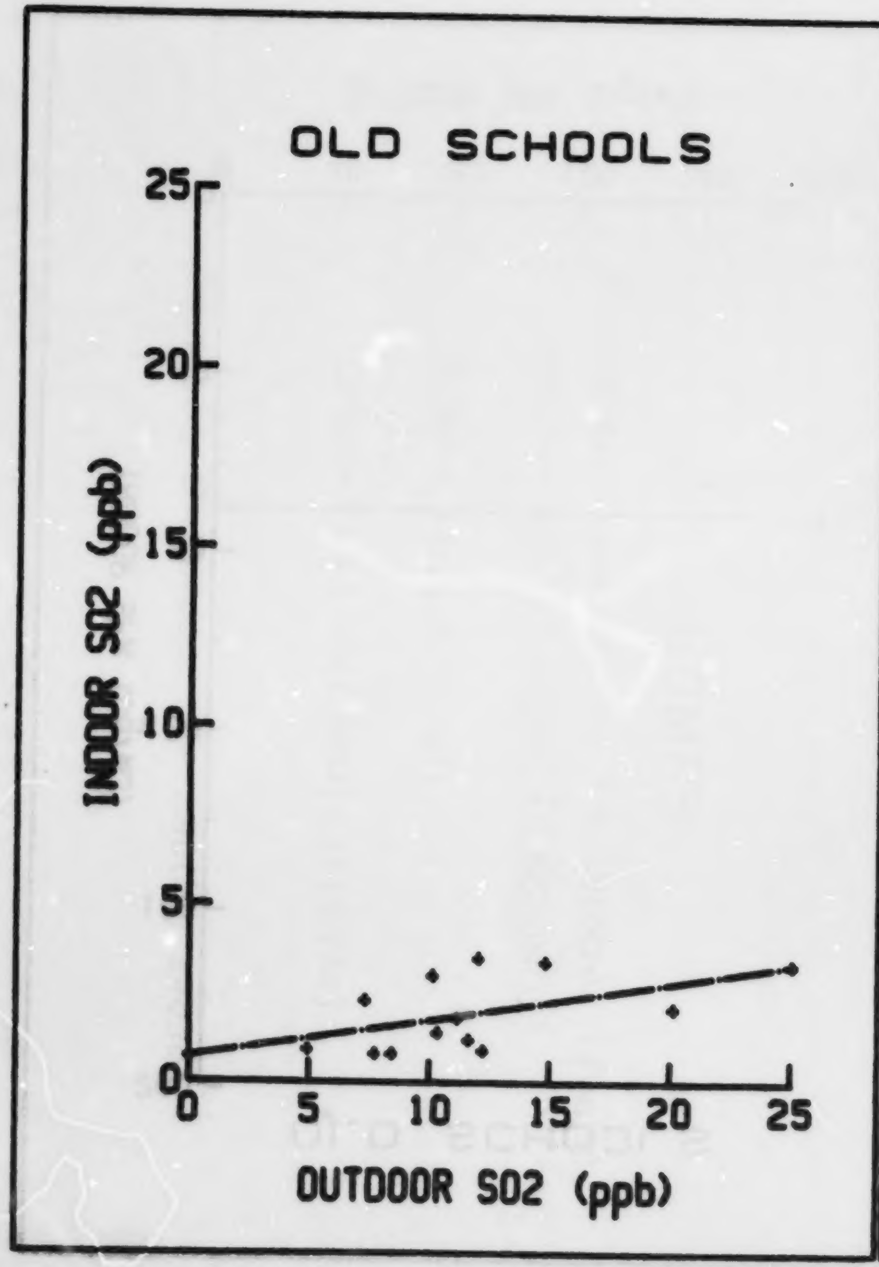
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INDOOR SO2 (ppb)
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5
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25
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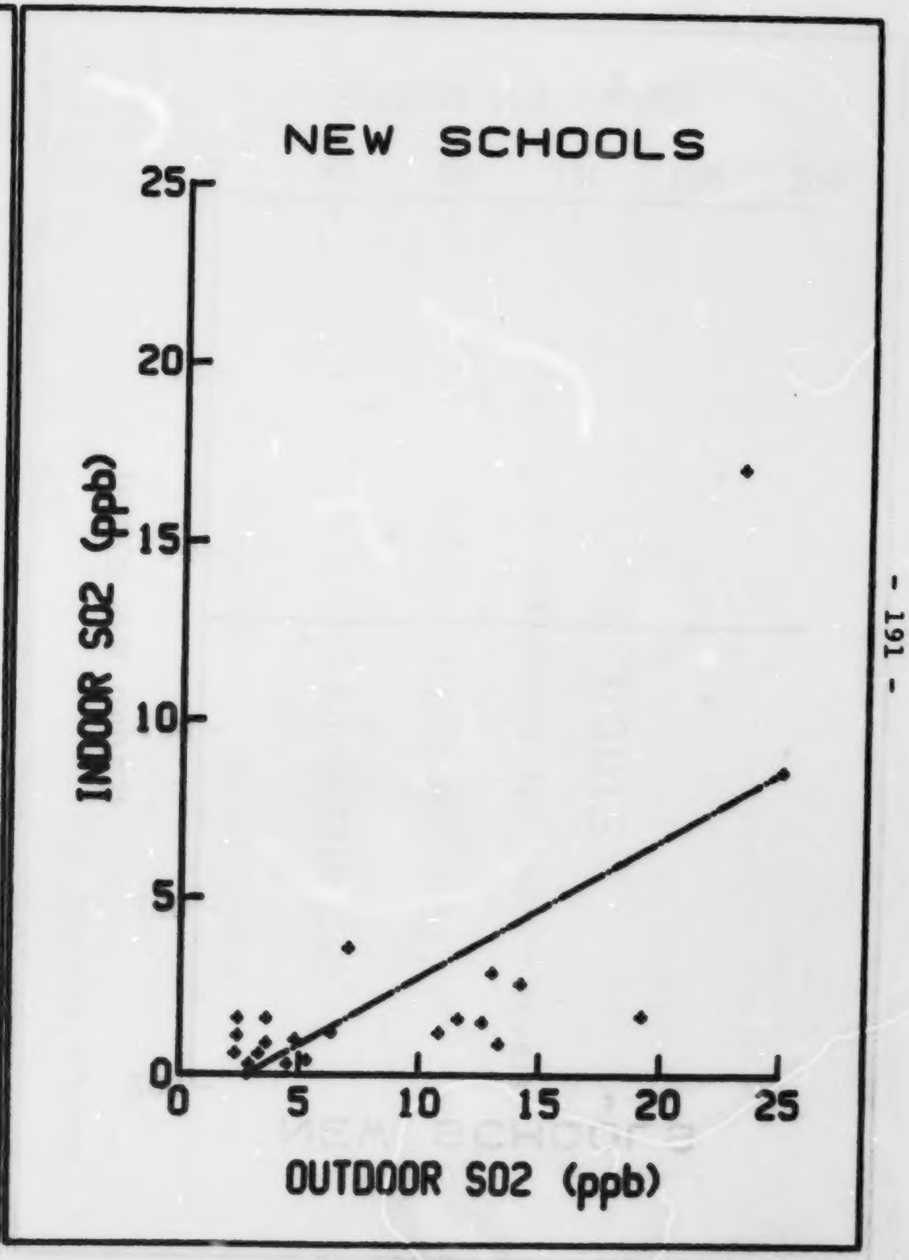
25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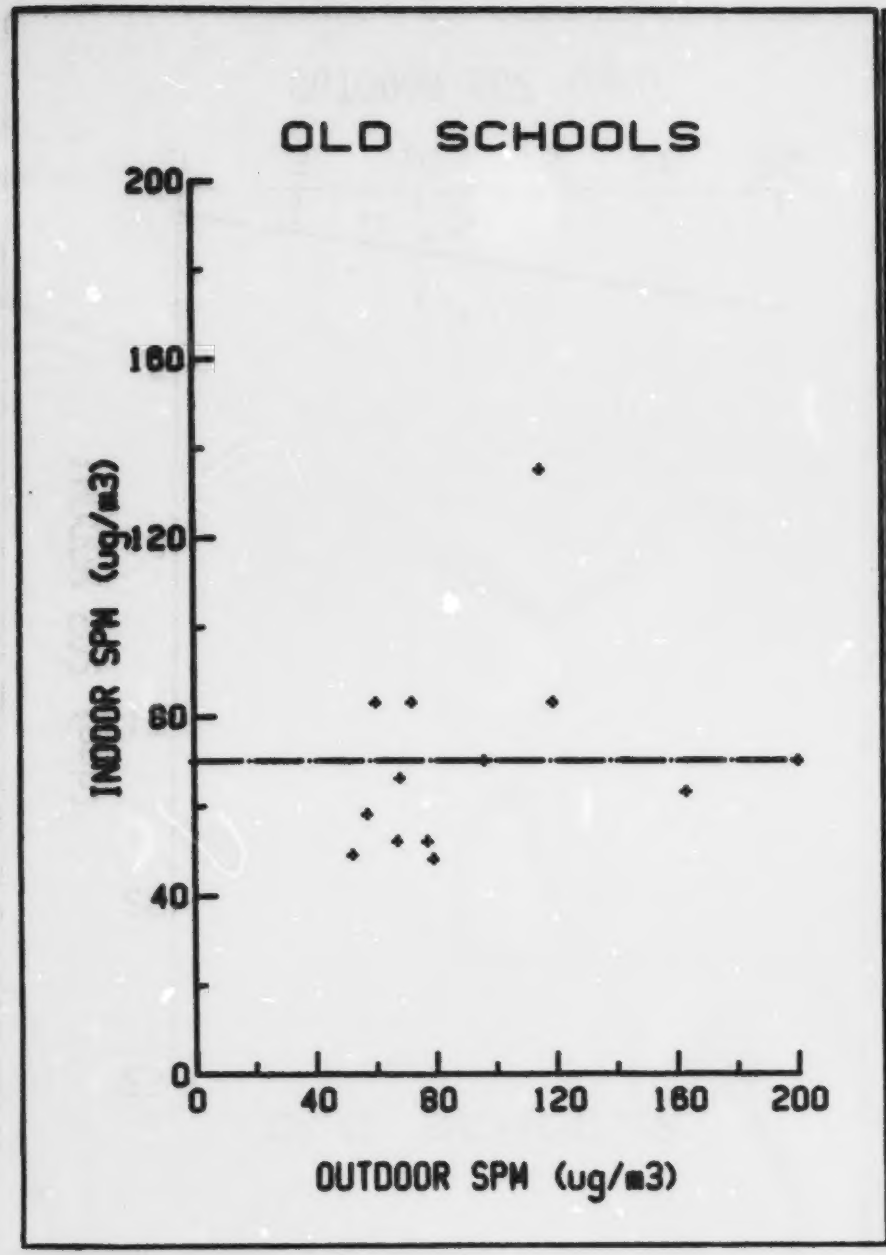
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120
60
40
0
INDOOR SPM (ug/m3)
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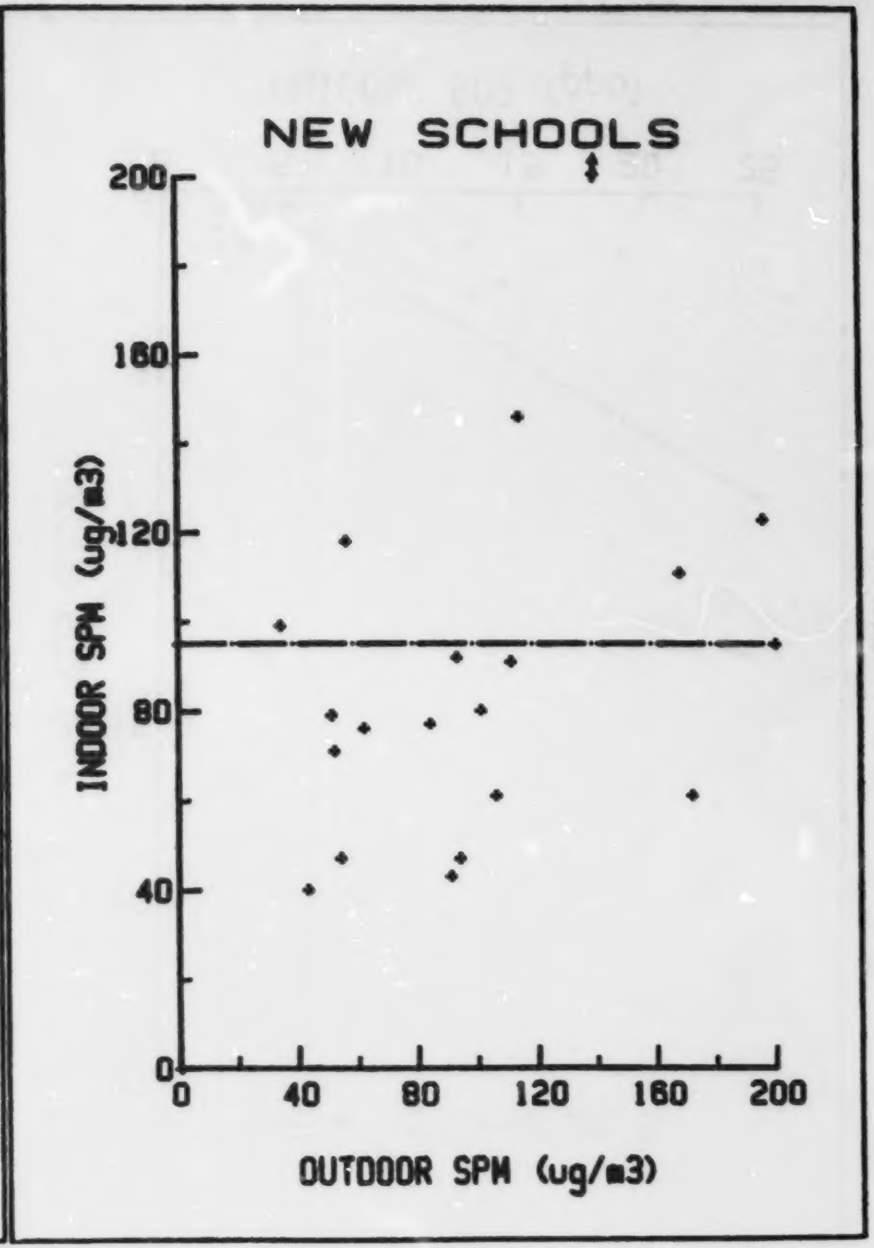
40
80
120
160
200
OUTDOOR SPM (ug/m3)


NEW SCHOOLS
200
160
120
80
40
0
INDOOR SPM (ug/m3)
0
40
80
120
160
200
OUTDOOR SPM (ug/m3)

SCHOOLS

Regression equations for indoor levels:

$INO2 = -1.0 + 0.80(ONO2)$

$ISO2 = -0.83 + 0.31(OSO2)$

ISPM is not fn of OSPM

(I=indoor,O=outdoor; heating season shows no effect.)

HOMES

Regression equations for indoor levels:

$INO2 = -0.5 + 17.6(Gas) + 0.86(ONO2)$

$ISO2 = 1.94 - 1.41(Heat)$

$ISPM = -24 + 1.13(OSPM) + 35(Smkr)$ *

*(I=indoor, O=Outdoor; *= heating season only.)*

COMBINED APPLICATION OF OZONE AND CHLORINE OR CHLORAMINE TO REDUCE PRODUCTION OF CHLORINATED ORGANICS IN DISINFECTION OF HIGH DOC DRINKING WATERS

J.L. Robertson[1]

ABSTRACT

The application of ozone as the primary disinfectant was investigated on water supplies in Ontario containing high concentrations of dissolved organic carbon (DOC), as a means to reduce elevated levels of trihalomethanes and other chlorinated organics. Laboratory and pilot plant studies demonstrated that the use of ozonation to replace pre-chlorination resulted in substantially reduced THM and NPOX concentrations with subsequent post-chlorination, as compared to conventional pre- and post-chlorination. THM and NPOX production were found to be negligible if ozonation was combined with chloramination as the secondary disinfectant. Batch tests showed that these conclusions applied to another high DOC water supply, although the THM and NPOX production per unit of DOC varied between water supplies. This demonstrated the need for pilot studies on individual water supplies to develop data for process design. Preliminary cost estimates for various sizes of ozonation plants were prepared to assess the general economics of adding ozonation to existing water treatment plants. The study concluded that ozonation, combined with controlled post-chlorination or chloramine application, represented an effective strategy for control of THM and NPOX in high DOC waters while maintaining bacteriological quality and providing colour reduction.

Keywords: ozonation, chlorination, chloramination, trihalomethanes, chlorinated organics, disinfection, drinking water, cost estimates

[1] IEC BEAK CONSULTANTS LTD.
6870 Goreway Drive
Mississauga, Ontario
L4V 1P1
(416) 671-2600

INTRODUCTION

The role of chlorination in drinking water disinfection is being re-examined as a result of the discovery of trihalomethanes in Dutch drinking water by Rook (1974). Subsequently, trihalomethanes were measured in North American drinking water by Bellar and Lichtenberg (1974), by Symons et al. of the USEPA (1975), by Nicholson et al. of the Ontario Ministry of the Environment (1976) and Health and Welfare Canada (1977). Ontario MOE data (1977) indicated that ten of forty-two municipalities had total trihalomethane levels in their drinking water in excess of 100 ug/L in the summer months, as measured by the direct aqueous injection (DAI) method. Chloroform has been declared a potential carcinogen and bromo-chloro-methanes have been found to be mutagenic. This resulted in the promulgation of a 100 ug/L limit on total trihalomethanes in drinking waters by the U.S. EPA in the 29 November 1979 Federal Register.

Conventional water treatment utilizes chlorination for a variety of purposes including:

- primary disinfection of raw water
- taste and odour control (occasionally through super-chlorination)
- control of bacterial and algal growths within clarifiers and filters
- colour removal
- destruction of ammonia (breakpoint chlorination)
- provision of distribution system residual

Where the raw water supply contains high concentrations of organic carbon (eg. humic materials) or high algal concentrations, chlorination of the raw and treated waters will result in the formation of trihalomethanes and other organo-halogen compounds. As many of these compounds represent a potential health hazard, their minimization or elimination is considered to be beneficial to public health.

There are a variety of water treatment options available for preventing the formation of part or all of the THM and organo-halogen compounds. These include the following options singly or in combination:

- elimination of pre-chlorination or change in point of application
- THM precursor removal by coagulation and/or direct filtration
- use of alternate disinfectants such as ozone, chlorine dioxide, or chloramine
- precursor removal by GAC or ozone/GAC.

Where raw water quality is such that pre-disinfection cannot be eliminated, use of alternate disinfectants is a feasible method for minimizing or eliminating THM and TOX formation. This will occur with raw waters having high DOC and poor bacteriologic/algal quality.

IEC BEAK has completed a study for the Ontario Ministry of the Environment to investigate the technical and economic feasibility, and some of the public health aspects, of ozonation to reduce the concentrations of trihalomethanes in drinking water by replacing chlorine in the primary disinfection process. The project involved the use of both bench and pilot scale water treatment systems to evaluate ozone application for the primary or pre-disinfection step, and considered both chlorine and chloramine for the secondary or post-chlorination step.

A detailed description of the experimental program was presented in 1982, at the Technology Transfer Conference No. 3. In this paper, some of the important technical aspects of the project are reviewed, together with cost estimates for "add-on" ozonation systems, and some practical considerations for the application of ozonation.

PROJECT DESCRIPTION

As indicated, a detailed description of the project has been already presented. The overall approach to the project is described below to provide background information for the presentation of the key technical results of the project.

Objectives

The specific objectives of this program were:

- to demonstrate the effectiveness of ozonation as a water tretment step for controlling the production of trihalomethanes and other chlorinated organics in water treatment.

- to investigate the practical methods which might be employed in water treatment plants to reduce the formation of undesirable by-products.

- to identify as many of the organic by-products of ozonation of surface water supplies as possible.

- to assess the public health effects of ozonation with respect to disinfection and mutagenic activity.

- to assess the add-on cost of providing ozonation at existing water treatment plants.

Approach

The project was undertaken in two phases to examine the feasibility of using ozone in Ontario for the treatment of drinking water supplies.

Phase I consisted of the following major tasks:

- **literature review**
- **bench scale batch experiments on application of ozone followed by chlorine or chloramine**
- **mutagenicity assessment of the organic by-products of ozonation, ozonation/chlorination, and ozonation/chloramination**
- **identification of organic by-products by GC/MS and HPLC/UV fluorescence.**

Raw waters from three Ontario municipalities were used in the Phase I experimental work to evaluate the potential for both the formation and the minimization of trihalomethanes and other chlorinated organics in the disinfection process. Brantford and Lindsay were selected as they represented water supplies from an industrialized area, and rural area respectively, with high DOC. Toronto was also selected because it draws water from Lake Ontario which serves as a water supply for a large portion of Ontario's population and had a low DOC.

In Phase 2, the following major tasks were completed:

- literature review
- seasonal pilot-scale testing of ozone followed by chlorine or chloramine to confirm the results of Phase 1 under field conditions
- preparation of cost estimates for providing add-on ozonation at existing water treatment plants.

The demonstration pilot plant was set up at Lindsay, Ontario. Lindsay municipal water is drawn from the Scugog River, which flows from a relatively marshy area. The DOC is high, typically 8 to 12 mg/L, due to the presence of natural organic material in the raw water. Typically, water temperatures may vary from freezing to 25°C during the year. Colour in the raw water typically varies from about five units in the winter to as high as 50-60 units in the summer. High bacterial and algal counts occur during the summer months with some taste and odour.

The factors which were evaluated in both the bench scale and pilot scale programs had been determined from the literature and were similar for both programs. These factors, together with the pilot plant conditions, are presented below:

Factor	Levels (Pilot)
o DOC	o normal variation - 6 to 12 mg/L
o ozone dosage	o 4 levels - 0, 2.0, 7.5, 20 mg/L
o delay time between ozonation and subsequent chlorine residual application	o 3 levels - 3, 6, 24 hours
o storage time after chlorine residual application	o 3 levels - 4, 24, 72 hours
o type of residual applied	o 2 levels - hypochlorite or chloramine
o seasonal effects of water quality including temperature	o 3 seasons - winter, summer, fall

For the pilot scale test program, these factors were set up in incomplete factorial experimental designs for each season to facilitate statistical analysis. Details of the approach were published in the MOE Technology Transfer Conference No. 3 proceedings.

TECHNICAL ASPECTS OF OZONATION

Disinfection with Ozone and Hypochlorite

Statistical analysis of the experimental data from the pilot plant ozonation studies yielded the following results at the 95% confidence level.

- delay time between ozonation and subsequent chlorine residual application had no significant effect on THM production
- storage time after chlorine residual application had no significant effect on THM production
- the application of ozone as the primary disinfectant significantly reduced THM production although dosage had no significant effect in the range of 2-20 mg/L of ozone applied.

As a consequence of the first two results, it was possible to pool the THM data from all test runs at each applied ozone dosage to obtain the mean chloroform production data shown in Table 1. The data from Table 1 are presented graphically in Figure 1.

Table 1 and Figure 1 illustrate that the use of pre-ozonation reduces chloroform production but that ozone dosage had little effect on the magnitude of the reduction. Up to 60% reduction in chloroform could be achieved by replacing conventional pre-chlorination/post-chlorination with pre-ozonation/post-chlorination, when a 30 minute chlorine residual was applied. Figure 2 presents the chloroform data for the measured raw water temperatures to illustrate the reductions in chloroform production possible from the elimination of pre-chlorination and its replacement by pre-ozonation.

The lack of effect of delay time and storage time on chloroform apparently resulted from the high DOC and associated high chlorine demand. While each sample contained a residual of 0.5 mg/L of free chlorine after 30 minutes, this residual was partially exhausted and the remainder converted to chloramine residual, within the shortest storage time of 4 hours. A total chlorine residual of 0.1-0.2 mg/L remained in all samples up to the longest storage time of 72 hours. In effect, the amount of free chlorine was insufficient to meet the demand from 4, 24, or 72 hours storage thus limiting the amount of chloroform produced.

This has significant implications for the practical application of ozonation to provide properly disinfected water from high DOC raw water sources having high coliform counts and potential taste and odour problems. In previous research, THM formation potential was determined by the addition of an excess of free chlorine and observing the THM production with time. It was in this manner that previous researchers determined that the effectiveness of ozonation for reducing the production of THM decreased with increasing delay time between pre-ozonation and post-chlorination.

In practice, however, the delay time is dependent on the amount of treatment required to purify the raw water (eg. coagulation and filtration) and cannot be varied appreciably. The results of this study demonstrate that limiting the free chlorine available for reaction, prevents the increased THM production resulting from increased delay time and storage time observed in other studies. Provided that the high bacteriological quality of the treated water can be maintained, ozonation followed by controlled or limited chlorine application represents an effective strategy for reduction of THM. This could be achieved by the application of a free chlorine residual with a short contact time prior to addition of ammonia to form chloramine. This would provide a lasting residual and would not react readily to form THM or other chlorinated organics.

The NPOX data presented in Table 2 show that low applied ozone dosages (2 mg/L) reduced NPOX production by 50% to 60%, but as higher ozone dosages were applied NPOX production increased. This would be expected if it was assumed that the destruction of existing organic sites for chlorination resulted from low ozone dosages, and was followed by generation of new sites as a result of the action of higher dosages on the organic matrix. This assumption implies a sharp decrease in chlorine demand at low applied ozone dosages followed by a gradual increase in chlorine demand with increasing applied ozone dosages. The chlorine demand data showed such a pattern. When NPOX was plotted against chlorine demand, a significant linear relationship was found.

Disinfection with Ozone and Chloramine

A parallel set of experiments using chloramine as the secondary disinfectant following ozonation showed that no chloroform or other THM were produced during post-chloramination. A fresh chloramine solution prepared with distilled water was used to produce a 0.5 mg/L total chlorine residual in the ozonated water samples. In practice, low concentrations (< 10 ug/L) of THM may be formed during the addition of chlorine to a water containing natural organics used as feedwater to the chlorinator.

Measurement of NPOX production in the same experiments with chloramine showed that the use of chloramine instead of chlorine reduced the NPOX levels to < 10 ug/L.

Loss of residual during storage was not a problem with chloramine as it had been with hypochlorite. Starting at a total residual of 0.5 mg/L after 30 minutes, total residuals remaining at 4 and 24 hours were in the range 0.15 to 0.35 mg/L. This is adequate for bacteriostatic purposes (Rich, 1963). Residuals of 0.05 to 0.15 mg/L after 72 hours storage were measured indicating a need for higher dosages to provide adequate residual protection.

For the purpose of controlling THM and NPOX production in high DOC water supplies, the combination of pre-ozonation and post-chloramination appears to offer the opportunity to minimize or eliminate these classes of compounds. When considered in light of control of the taste and odour and colour removal by ozonation, the advantages appear even greater.

Effect of Water Supply

Batch ozonation tests were performed on Lindsay raw water as well as on raw water from the cities of Brantford and Toronto. The purpose of these tests was to determine the effect of water supply on THM and NPOX production. Chlorine and chloramine dosages and application procedures were similar to those used in the pilot scale testing. Ozone was applied according to an ozone/DOC ratio to ensure that equivalent ozone conditions were used for each water supply. The ratios used were 0.2, 0.75 and 2.0, which corresponded to the 2, 7.5 and 20 mg/L dosages applied at Lindsay in the pilot testing. The pooled THM and NPOX data are presented in Tables 3 and 4 respectively.

The THM Production from DOC (ug/mg) data were calculated by summing the ug/L concentrations of the four trihalomethanes $CHCl_3$, $CHCl_2Br$, $CHBr_2Cl$ and $CHBr_3$ and dividing by the DOC in mg/L. Several observations may be made as follows:

- different water supplies appeared to produce different quantities of THM per unit of DOC when subjected to chlorination alone and ozonation/chlorination,
- ozonation/chlorination produced less THM than did chlorination alone,
- chloramine produced negligible THM and very low concentrations of NPOX in all water supplies, either alone or in combination with ozone
- ozonation/chlorination appeared to produce less NPOX than did chlorination alone.

The differences in production of THM and NPOX in different high DOC water supplies demonstrate the importance of conducting pilot tests prior to design of an ozonation facility, to determine the extent of THM and NPOX reduction achievable.

Public Health and Aesthetics

Total and fecal coliform counts were performed on samples of raw and pre-ozonated water collected during the pilot plant testing. Table 5 presents the mean total coliform data for raw and pre-ozonated water grouped by season and ozone dosage. No data on viruses were collected.

It is evident from Table 5 that pre-ozonation at all dosages resulted in effective destruction of total coliform. It appears, therefore, that the turbidity present in the raw water (1-10 JTU) did not adversely affect the disinfection of the raw water by ozone. Complete destruction of fecal coliforms was also observed.

True colour measurements were made with a comparator in all three seasonal surveys. On the basis of the comparator results presented in Table 6, it is apparent that significant reduction of true colour was obtained through pre-ozonation as has been observed in previous work.

The data for March indicated greater than 75% removal of colour, to a level below the MOE drinking water criterion of 5 units at all ozone dosages. However, in July colour could be reduced only by 50% to 65% to between 10 and 15 units depending on ozone dosage. Even at 20 mg/L of ozone the maximum colour removal averaged 65% and left residual true colour of 10 units in the ozonated water. This is consistent with observations by previous researchers who observed that in highly coloured natural waters, a refractory portion of the colour remained after ozonation.

The results of the organic identification work by GC/MS and HPLC showed that ozonation produced a wide variety of new compounds. Most of these may be rationalized in terms of oxidative cleavage of the natural humic materials in the raw waters. Higher concentrations of these materials resulted from higher ozone dosages, as expected, and also from higher DOC concentration in the raw water. Lindsay water, which had the highest DOC, also yielded the largest number of ozonation by-products. Many of the by-products were ketones, aldehydes or organic acids presumably formed from the cleavage of carbon-carbon double bonds by the classical Criegee mechanism. Several aromatic carboxylic acids were found, as well as many substituted phenols. These presumably were formed by cleavage of the natural aquatic humic materials.

In general, the compounds identified were in the low parts-per-billion concentration range or less, and their combined concentrations represent a small fraction of the largely uncharacterized matrix of organic carbon compounds found in natural waters. Most of the organic ozonation products found are similar to products obtained by natural oxidation processes and are probably innoccous.

The results of Ames' salmonella testing for bacterial mutagenicity showed essentially no mutagenic activity from the organics isolated by a vacuum rotary evaporation procedure. The rotary evaporation procedure recovers the non-volatile organic materials from water which have boiling points greater than 100°C. This represents in excess of 90% of the organic matter present in water supplies. The results in this study have been interpreted to indicate that this large fraction of the organics in raw and treated waters are not mutagenic under the test conditions applied in this study.

ECONOMICS OF OZONATION

To provide realistic cost estimates for ozonation systems in Ontario, a variety of plant sizes and ozone dosages had to be considered. Application of pre-ozonation most likely will be considered for water supplies having high DOC concentrations and hence high THM's, taste and odour problems, excessive colour and/or low bacteriological quality. On the basis of the pilot testing conducted in this study, and from literature information on taste and odour, ozone dosages of between 2.0 and 7.5 mg/L will provide the desired treated water quality. Consequently these two dosages were used to estimate costs for ozonation systems which could be added on to existing water treatment facilities.

Capital and operating costs for intermediate dosages can be estimated from the range of costs provided.

Water treatment plants in Ontario range from less than 400 m^3/day (100,000 USgpd) to over 200,000 m^3/day (50 MUSgpd). Because of the capital costs of ozonation systems, very small municipalities are unlikely to consider ozonation except in extreme circumstances. Consequently, plant sizes of 1900 m^3/day (500,000 USgpd), 19,000 m^3/day (5.0 MUSgpd) and 190,000 m^3/day (50 MUSgpd) were chosen representing populations of approximately 5,000, 50,000 and 500,000.

Detailed capital and operating costs for these plant sizes were calculated based on "add-on" ozonation systems for hypothetical existing plants. As such, these estimates should not be considered applicable to all water treatment plants of similar capacity. All costs are in 1982 dollars.

Capital costs included direct costs such as building construction, contact tanks, water piping, ozone process equipment, electricals and overhead costs including design and site engineering, contract administration, bonds and permits insurance and contingency. It was assumed that land would be available at existing plants. Budgetary costs were obtained for major items from suppliers and contractors.

Annual operating costs were calculated and included process energy, building energy, labour and maintenance material. Process energy (electrical) was assumed at $0.03/kw-hr.

Total annual costs were calculated from the capital and operating costs as shown in Table 7. These were then converted to a cost per cubic metre of water treated for comparison to existing water rates for various municipalities shown in Table 8.

Cost of "add-on" ozonation ranged from $0.06/$m^3$ for the 7.5 mg/L dose at the 1900 m^3/day plant, to $0.007/$m^3$ for the 2.0 mg/L dose at the 190,000 m^3/day plant. Water rates appeared to be independent of plant size for the municipalities surveyed, so an average water cost of $0.29/$m^3$ ($1.08/1000 USgal) was used. On this basis, addition of ozonation would increase water rates by 20 to 25% for a small plant to 2.5 to 4.5% for a large plant, assuming the cost is passed on direct. For municipalities, such as Lindsay, where there is a significant annual cost for chlorine, (dosages as high as 10 mg/L) a credit would be realized from the displaced chlorine consumption. This credit would pay for the ammonia needed for chloramination as well as help to offset the annual cost of ozonation.

APPLICATIONS OF OZONATION

Where raw water quality is such that pre-disinfection cannot be eliminated, use of alternate disinfectants is a feasible method for minimizing or eliminating THM and TOX formation. This study examined the use of ozone as an alternate primary disinfectant in combination with both chlorine and chloramine as secondary disinfectants.

As ozonation provides no residual for bacteriostatic action in the distribution system a persistant residual is applied after treatment to last through the distribution system. The results of the pilot studies presented in this report indicate that either short-term chlorination or chloramination could provide the necessary residual and reduce or eliminate THM and chlorinated organics. Selection of short-term chlorination or chloramination for final disinfection will depend on:

- level of THM's achievable by short-term chlorination
- organic nitrogen content of treated water available to provide combined chlorine residual if chlorination is used.

Ozonation has been shown in this study to provide substantial reduction in THM formation by elimination of the pre-chlorination step and by destruction of precursor materials. The data showed that the reductions in THM concentrations achievable with ozonation are largely unaffected by ozone dosages in the range 2-20 mg/L, although there is a trend to lower THM concentrations with increasing ozone dosage.

The decision to use ozonation should be based on consideration of a number of factors and how these are related to the specific water treatment needs and economic considerations at any given water treatment plant. For example, pre-ozonation will reduce or prevent the formation of THM and NPOX when followed by controlled chlorination or chloramination; pre-ozonation will simultaneously destroy or reduce colour depending on the water supply, and provide excellent bacterial destruction. The

City of Monroe, Michigan has had excellent results using pre-ozonation to control taste and odour problems. The actual dosages applied will depend on the purpose of using ozonation and will need to be evaluated in pilot scale tests. In general, however, factors other than THM control will influence dosage selection.

The evidence available from this study and the literature, indicates that ozonation itself generates fewer potentially harmful by-products than chlorination, and appears to destroy most potentially harmful compounds when applied in concentrations used in water treatment. The Salmonella assay results from this study indicate no mutagenic activity of the non-volatile organics in ozonated water, while the reduction of the THM indicates a beneficial health effect. It should be emphasized that the Ames' test data presented in this report represent only the first step in the rigorous procedures needed for risk assessment.

Based on the results presented, complete elimination of THM and NPOX could be achieved through the use of pre-ozonation and post-chloramination. The literature and the Ames' test results in this study indicate that the ozonation/chloramination system would provide the maximum public health benefit in terms of good micro-biological quality and almost complete elimination of bacterial mutagenic agents such as THM and other chlorinated organics.

Because of the capital costs involved in applying both ozone and chlorine dioxide, extensive bench tests and full-scale pilot trials are recommended to determine if acceptable THM and TOX levels can be achieved by modifications of the conventional processes first. If taste, odour and colour removal are also necessary, then alternate disinfectants must be considered at the outset to achieve control of these problems as well as THM and TOX. In general, high DOC raw waters will have colour taste or odour problems associated with them in the warmer months of the year.

Extensive testing programs are recommended to establish the most feasible approach to achieving THM and NPOX removal, while maintaining treated water quality. Tests should be undertaken to establish which of the options for THM and NPOX removal is the most economical, is technically feasible and meets drinking water quality objectives.

When water quality considerations dictate the use of alternate disinfectants, pilot scale trials of ozonation and chlorination/chloramination should be undertaken, similar to those presented in this study.

The pilot trials should be undertaken under "worst case" raw water quality conditions, as these will result in the maximum THM production for comparison to drinking water quality objectives. Worst case conditions would occur mainly in mid-to-late summer when water temperature and DOC are at maximum.

Although no effect was observed for delay time between ozonation and post-disinfection at Lindsay, the delay time used in the tests should be equivalent to that which would occur in the full-scale facility. Similarly, the storage time after chlorination/ chloramination should be based on the retention period in the water distribution system. In this manner realistic THM and TOX data will be obtained on which to base decisions regarding choice of short-term chlorination with or without subsequent ammonia addition, or direct addition of a chloramine residual.

CONCLUSIONS

The following conclusions have been drawn from the study:

1. Ozonation has been shown to provide a substantial reduction in THM and NPOX production when combined with controlled post-chlorination. However, the reduction was largely independent of applied ozone dosage in the range 2-20 mg/L.

2. The decision to use ozone may be based on a need to reduce THM concentrations for public health reasons, but the selection of the applied dosage may be based on other factors such as destruction of micro-organisms, taste and odour control, colour removal and economic considerations.

3. As chloramine produced substantially lower THM and NPOX concentrations when applied as the secondary disinfectant, it would be the disinfectant of choice to provide a distribution system residual.

4. The differences in THM and NPOX production in different water supplies make pilot scale testing an important part of the selection of design criteria for the application of ozone to drinking water.

5. For water supplies containing high DOC (elevated THM), colour and bacterial/algal counts, "add-on" ozonation can deliver high quality treated drinking water at a 20-25% increase in cost to the consumer.

TABLE 1

Mean Chloroform Production by Hypochlorite-
Data Grouped by Ozone Dosage and Season

Nominal Applied Ozone Dosage	Mean Chloroform Production (ug/L)		
	March (5°C)	July (26°C)	Sept. (13°C)
0(Pre NaOCl)	-	320±33.6(9)	319.6±40.1(8)
0	87.6±31.3(2)	253±38.8(6)	186.4±16.8(6)
2	54.1±31.3(2)	173±38.8(4)	132.0±18.5(4)
7.5	53.2±13.2(7)	175±27.9(7)	125.6±14.6(6)
20	39.2±7.4(4)	154±16.4(4)	105.3±29.8(4)

Note: Data presented as Mean ± Standard Deviation (No. of data points in mean)

TABLE 2

NPOX Concentrations: Data Grouped by Ozone Dosage and Season

Nominal Applied Ozone Dosage (mg/L)	Mean NPOX Production (ug/L) March[1]	July[2]	September[2]
0	152±80(2)	153	52
2.0	60±55(4)	88	20
7.5	100±33(7)	65	45
20	137±60(4)	112	35

[1] Data presented as Mean ± Standard Deviation (Number of data points in mean)

[2] Data are for analysis of random sample at each ozone dosage

TABLE 3

THM Production from DOC

	DOC (mg/L)	THM Production from DOC (ug/mg) Chlorinated	Ozonated/Chlorinated
a) Hypochlorite			
Brantford	6.1	28.5	18.3
Lindsay	10.5	15.4	11.4
Toronto	2.7	14.1	8.4
b) Chloramine			
Brantford	6.1	0.0	0.0
Lindsay	10.5	0.0	0.0
Toronto	2.7	0.0	0.0

TABLE 4

NPOX Production from DOC

	DOC (mg/L)	NPOX Production from DOC (ug/mg)	
		Chlorinated	Ozonated/Chlorinated
a) Hypochlorite			
Brantford	6.1	54.2	21.0
Lindsay	10.5	24.9	18.8
Toronto	2.7	-	-
b) Chloramine		Chloraminated	Ozonated/Chloraminated
Brantford	6.1	10.7	7.4
Lindsay	10.5	10.8	6.5
Toronto	2.7	-	-

TABLE 5: COLIFORM REDUCTION IN PHASE 2 PRE-OZONATION

Nominal Applied Ozone Dosage (mg/L)	Mean Total Coliform Counts (per 100 ml)					
	March		July		September	
	Raw	Ozonated	Raw	Ozonated	Raw	Ozonated
2.0	8	3	-	-	269	1
7.5	15	3	-	-	256	0
20	9	3	-	-	142	0

TABLE 6: TRUE COLOUR REMOVAL BY OZONATION

Nominal Applied Ozone Dosage (mg/L)	Mean Total Coliform Counts (per 100 mL)					
	March		July		September	
	Raw	Ozonated	Raw	Ozonated	Raw	Ozonated
2	20	5	30	L5	5	5
7.5	20	5	30	L5	5	5
20	20	5	30	L0	5	5

TABLE 7: TOTAL ANNUAL COSTS OF OZONATION SYSTEMS

Plant Size (m^3/day)	Ozone Dosage (mg/L)	Capital Cost (\$1000)	Ann. Amort. Capital Cost (\$/yr)	Ann. Oper. Cost (\$/yr)	Total Ann. Cost (\$/yr)	Unit Cost (\$/m^3)
1,900	2.0	230	22,650	10,600	33,250	0.048
	7.5	285	28,000	14,700	42,700	0.062
19,000	2.0	615	60,150	22,000	82,150	0.012
	7.5	1210	118,500	54,700	173,150	0.025
190,000	2.0	3370	330,200	155,800	486,000	0.007
	7.5	6640	650,600	496,000	1,146,500	0.017

TABLE 8: TYPICAL COSTS OF MUNICIPAL WATER TO CONSUMER*

Municipality	Plant Size (m^3/day)	Plant Size (MUSGPD)	Cost ($/m^3)	Cost ($/1000 USgal)
Coniston	31,800	8.4	0.22	0.83
West Toronto	386,000	100	0.34	1.29
Goderich	4,400	1.2	0.28	1.08
Lindsay	8,200	2.2	0.37	1.38
Ottawa	350,000	92	0.28	1.06
Port Dover	3,500	0.94	0.23	0.88
Thunder Bay	38,200	10	0.35	1.31
Windsor	286,000	76	0.22	0.83
		Mean	0.29	1.08

* Based on use of 24,000 USgal during billing period for industrial consumer in 1982

FIGURE 1

EFFECT OF PRE-OZONATION ON CHLOROFORM PRODUCTION IN POST-CHLORINATION

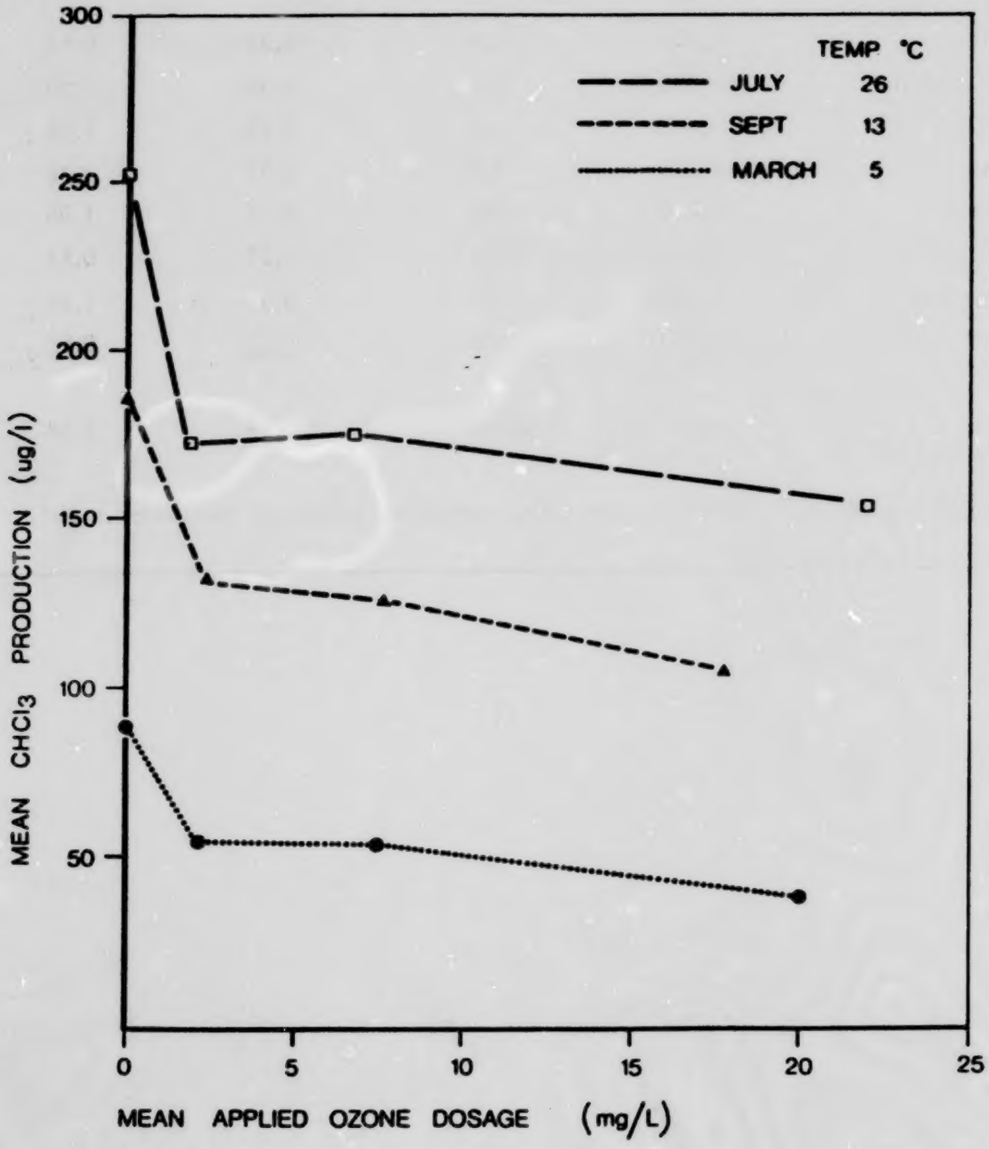
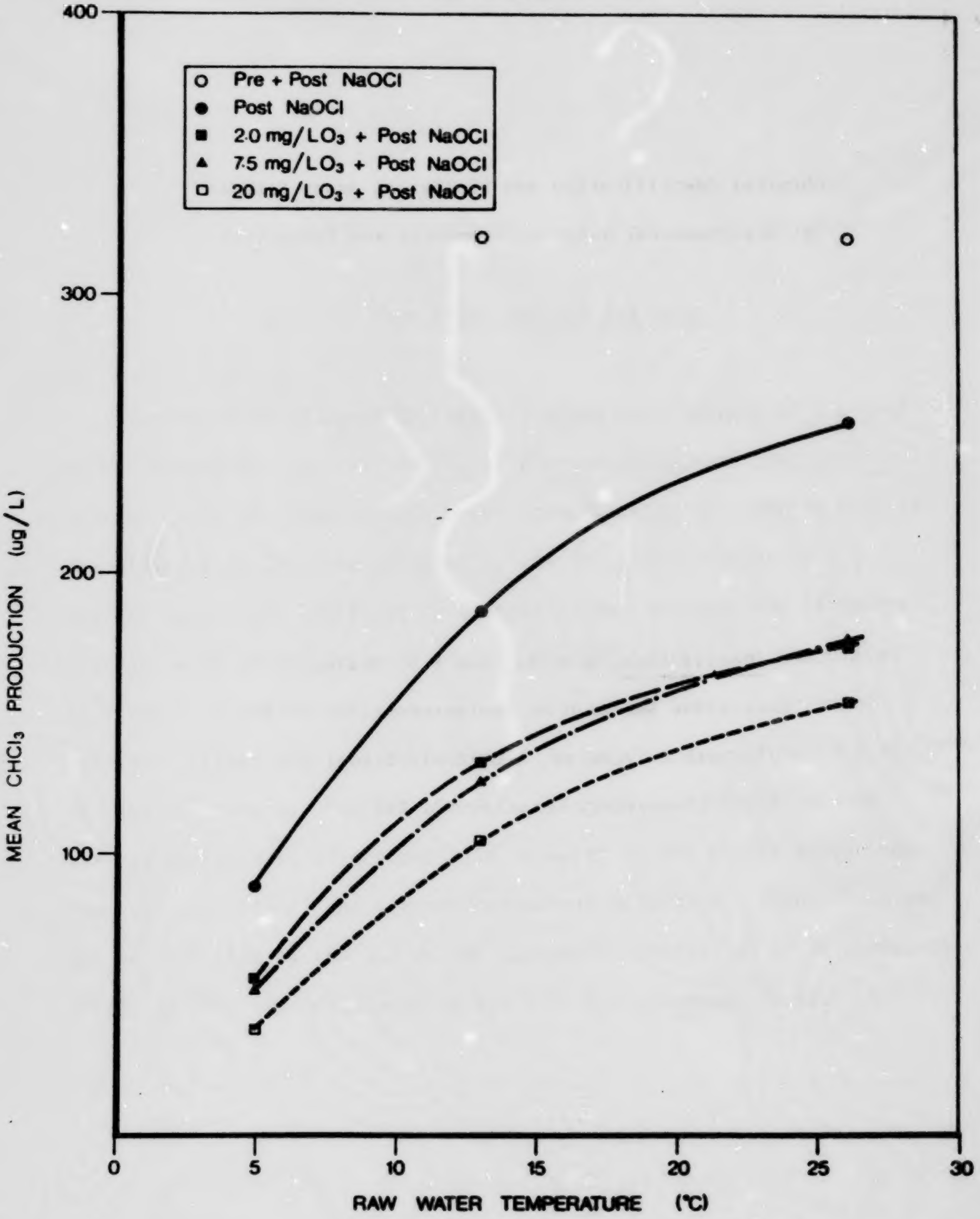


FIGURE 2

EFFECT OF SEASONAL CHANGES ON CHLOROFORM PRODUCTION



Chemical Identification and Biological Assay Studies of Environmental Mutgens, Promoters and Inhibitors

Morris Katz, A.S. Raj and K.R. Sharma
Centre for Research on Environmental Quality
York University, 4700 Keele Street
Downsview, Ontario, M3J 2R3

ABSTRACT

Chemical Identification and Analysis

The studies of chemical identification and analysis of environmental pollutants involved polynuclear aromatic hydrocarbons (PAH) in water samples. The methods under investigation included removal of the organics in the water samples by solvent extraction or by C_{18} Sep-Pak cartridges, followed by elution of the adsorbed PAH (from the Sep-Pak) with tetrahydrofuran (THF). The THF extract was evaporated to a small volume in a Kuderna-Danish evaporator under a stream of nitrogen. After addition of acetonitrile to a sample volume of 2 ml, the analysis was carried out by HPLC, using reversed phase columns, various percentages of acetonitrile in water as the mobile phase under isocratic conditions and spectrophotometric detection. Identification was accomplished by the use of PAH standards, consisting of 16 compounds listed as priority pollutants by the U.S. Environmental Protection

Agency. Data recording was accomplished by a computing integrator. Mass spectra of PAH were also determined for identification purposes.

Water samples from the Welland River, collected at the steel bridges opposite the City of Welland and at Wellandport, were analyzed for eight PAH compounds. These were detected in much higher concentrations at Wellandport than in the samples at the City of Welland. The contaminants included the following carcinogens and mutagens:- benzo(a)pyrene, dibenz(a,c)anthracene, dibenz(a,h)anthracene, benzo(g,h,i) perylene and indeno(1,2,3-cd)pyrene.

A procedure has been developed to improve greatly the efficiency of extraction of PAH from water samples by C_{18} Sep-Pak cartridges involving the acidification of the water samples to pH 2 and prior activation of the Sep-Pak with THF. Recoveries of PAH ranged from over 80 to nearly 100 percent.

Biological Assay Studies

The biological studies of mutagens, potential inhibitors and promoters have been conducted by means of a three-tier system of assays, consisting of the Ames Salmonella typhimurium test, and the *in vivo* bone marrow micronucleus and abnormal spermhead assays on mice. These assays have proved useful in delineating the mutagenic activity of representative PAH, such as 7,12-dimethylbenz(a)anthracene (DMBA), benzo(a)pyrene (BaP) and other chemicals, acting singly and in pairwise combinations. The mutagenic response of pairwise mixtures of promutagens such as BaP and DMBA or the other strong PAH mutagens was non-additive. However, the combination of a direct-acting mutagen such as mitomycin C (MMC) and BaP, yielded an additive response. Moreover, the combination of the promutagens BaP and cyclophosphamide (CP) was found to be additive. CP induces a different enzyme system and metabolic pathway than that of BaP. Combinations of a direct-acting mutagen and a promutagen or of two promutagens that act by different enzymatic pathways (e.g., BaP and CP) were found to yield an additive clastogenic response in both somatic and germ cells.

A considerable number of complex phenols and antioxidants have been tested to determine their effectiveness as inhibitors of mutagenesis. Studies of inhibitors reported in the literature have been confined mainly to an assessment of their role in preventing or reducing neoplasia by carcinogenic agents. However, we have found that a number of such substances, e.g., butylated hydroxyanisole and cysteamine, which have been cited as effective inhibitors of chemical carcinogenesis, show no degree of inhibition whatever against the mutagens DMBA, BaP, CP and others, when tested by means of the in vivo bone marrow micronucleus assay. However, effective inhibition against the mutagenic effects of DMBA and BaP has been demonstrated by 7,8-benzoflavone, 5,6-benzoflavone, ascorbic acid, caffeic acid, ethoxyquin, disulfiram, α-tocopherol and β-sitosterol, corn oil and sunflower oil.

Preliminary studies have been conducted to determine the effects on PAH mutagens of promoters such as arochlor, phenobarbital and hexachlorobenzene, using the in vivo bone marrow micronucleus and abnormal spermhead assays.

I. CHEMICAL IDENTIFICATION AND ANALYSIS OF POLYNUCLEAR AROMATIC HYDROCARBONS IN WATER

The determination of polynuclear aromatic hydrocarbons (PHAs) and other organic compounds in water samples has usually been carried out by removal of organic material from the water using liquid-liquid extraction techniques (1,2,3,4,5), head space sampling techniques (6,7,8) or adsorption techniques employing solid adsorbents such as charcoal (9), Tenax (10), XAD-type resins (11) and polyurethane foam (12,13). Both XAD-4 aND XAD-8 in equal proportion were used in the column to extract organics of varying polarities from drinking water (14). More recently reversed-phase liquid chromatographic packing has been used to extract and concentrate trace levels of organics from water. May et al. (15) used a C_{18}-bonded phase packing for the extraction of hydrocarbons from water. Numerous reports have described the potential of reversed-phase packing for trace enrichment of organics from various aqueous samples (8,9,10,11,12). Traditional concentration and cleanup techniques employ multiple solvent extractions followed by solvent evaporation. These time consuming methods usually employ large amounts of solvents. There is, however, an urgent need for rapid methods because in many cases a large number of samples have to be analyzed. The technique of trace enrichment using a suitable column affords a simple means of simultaneous extraction, concentration and purification of trace amounts of organics from water samples in the same step. The present paper describes the feasibility of trace enrichment of water samples by commercially available Waters Sep-Pak C_{18} cartridges.

Concentrations of polynuclear aromatic hydrocarbons (PAHs) in drinking water sources and supplies have been found to range between a few nanograms to several hundred nanograms per liter (16,17,18). The techniques used to measure PAHs in environmental samples have been thin-layer chromatography, gas chromatography and GC-mass spectroscopy (19,20,21). However, each suffers certain inadequacies with respect to concentration, separation, detection or identification of PAHs. Separation of isomeric PAHs has been difficult with packed column GC analysis using both conventional and MS detectors (22,23). All these methods utilize liquid-liquid extaction, the traditional method of extracting and concentrating organic compounds from water which require several time consuming steps, large volumes of volatile organic solvents and considerable analytical skill. An alternative method is adsorption trapping, in which the organic compounds are extracted from a flowing sample by strong adsorption onto a solid matrix. Various adsorbents have been proposed as effective matrices for removal of organics from water (24,25,26).

More recently, reverse-phase octadecylsilane (ODS) chromatographic packing has also been used as a means of trace enrichment of organics from various aqueous samples including distilled water (27), drinking water (28,29), urine (30), chlorinated water (31), natural water (32) and waste water (33).

However, data on the efficiency of recovery and reproducibility of analytical methods based on adsorption trapping and trace enrichment of PAH vary widely with different solid matrices. It is advisable, therefore, to establish the isocratic conditions and extent of recovery of PAH for each particular type of solid adsorbent that is employed to trap such organics in water samples.

Instrumentation

Chromatography was conducted using Waters liquid chromatograph Model 204 with dual Model 6000A solvent delivery pumps, a model U6K universal injector, a Model 600 solvent programmer, Supelco LC-PAH reversed-phase analytic column and Waters radial compression separation system containing Z-module and a Radial-Pak PAH analytical cartridge column. Data recording was done on a Spectra Physics Model 4270 Computing Integrator. Detection was accomplished via Waters Model 440 UV detector at 254 nm.

Preparation of Standards and Samples

The PAH standards used in this study were obtained from Supelco containing 16 PAHs which comprise the U.S. Environmental Protection Agency (EPA) list of priority pollutants for water analysis. These compounds were obtained as solutions in methanol/dichlormethane 50/50 (V/V) and were diluted further, 1 ml to either 10 ml or 100 ml, in acetonitrile prior to use as standards. The stock solutions of standard PAHs were stored in a refrigerator. The synthetic samples of water were prepared by adding 1 ml of diluted standard PAH solution to 50 ml of high purity water from Millipores-Milli Q purification system.

Procedure

Each Waters Sep-Pak cartridge contains 0.35g of C_{18} porous (70 μm particle size) packing material (140 m^2 of surface area per cartridge). The Sep-Pak obviated hand-packing of non-disposable guard columns and thus reduced any accidental contamination and packing variations associated with this procedure. More importantly, Sep-Pak cartridges allowed the use of hand-held syringes instead of electrical pumps to pass through water samples for extraction of organics, thus improving portability for on-site sampling. These cartridges are made specifically to fit a Luer-lock tipped syringe for introduction of solution or solvents manually.

Prior to trace enrichment, the Sep-Pak cartridge was always activated by passing 10ml tetrahydrofuran, 10ml methyl alcohol followed by a rinse of water at pH 2. Then 50ml of synthetic water samples (1ml of PAH standard in 50ml of water) at pH 2 was passed through an activated Sep-Pak at a flow rate of 1 drop/sec. The Sep-Pak was dried by passing air through it until no water drops were observed. The adsorbed PAHs were eluted with 10ml of glass-distilled dichloromethane into a 25ml pear-shaped flask. Dichloromethane was removed completely from the extract with a stream of dry nitrogen by a rotary evaporator. One ml of glass-distilled acetonitrile was then added to the residue and 10 or 25µl of this sample was analysed by HPLC.

Runs were carried out under both isocratic and gradient conditions. Quantitation was accomplished by comparing the sample peak areas to those of standards obtained under identical conditions.

Results and Discussion

Figure 1 shows the chromatogram of 16 PAH standards obtained from Waters Z-module consisting of a Radial Pak PAH cartridge column. Table 1 lists the peak number of the PAHs in order of increasing elution time and their concentrations. Figure 2 shows the chromatogram of PAHs concentrated from synthetic water samples at pH 2 by Sep-Pak.

It was found that the extraction of PAHs from the synthetic water samples by Sep-Pak, without pH adjustment of the water, yielded a wide range of percent recovery. A similar trend was observed in the liquid-liquid extraction of the PAHs with dichloromethane from water samples at about pH 7.

Table 2 shows the percent recovery of PAHs obtained from the synthetic

TABLE 1: Retention Time of PAHs (Supelco Standard) and Their Concentration

PAH Compounds (in order of elution)			Retention (min)	Concentration (mg/ml)
1	Naphthalene	Nph	4.49	0.10
2	Acenaphthylene	Acelene	6.06	0.20
3	Acenaphthene	Ace	8.75	0.10
4	Fluorene	Fl	10.14	0.02
5	Phenanthrene	Phe	13.44	0.01
6	Anthracene	An	16.04	0.01
7	Fluoranthene	Ft	18.16	0.02
8	Pyrene	Py	19.12	0.01
9	Benz(a)anthracene	B(a)An	23.57	0.01
10	Chrysene	Chy	24.18	0.01
11	Benzo(b)fluoranthene	B(b)Ft	27.26	0.02
12	Benzo(k)fluoranthene	B(k)Ft	28.70	0.01
13	Benzo(a)pyrene	B(a)Py	29.60	0.01
14	Dibenz(a,h)anthracene	diB(a,h)An	32.14	0.02
15	Benzo(g,h,i)perylene	B(g,h,i)Per	32.64	0.02
16	Indeno(1,2,3-cd)pyrene	In(1,2,3-cd)Py	33.71	0.01

water samples when the pH was not adjusted to 2. It was found that the high molecular weight PAHs such as diB(ah)An, B(ghi)Per and In(1,2,3-cd)Py gave a low recovery by both Sep-Pak and liquid-liquid extractions, without pH adjustment. Several runs were carried out to investigate the recovery of B(ghi)Per in 50 ml of water (without pH adjustment) by both Sep-Pak and liquid-liquid extraction using dichloromethane. Similar experiments were performed with this PAH in water samples adjusted to pH 2 and the results indicated greatly improved recovery (see Table 2, footnote).

Clearly, both liquid-liquid extraction and Sep-Pak enrichment gave higher recovery percentages from synthetic water samples at pH 2 as shown in Tables 2 and 3. Similar observations were made by Eichelberger et al. (34) in their study of the recovery of sixteen PAH compounds on the EPA priority list when extracted from water samples adjusted to pH 2. However, they reported mean relative standard deviations in percent recovery of PAHs for packed column and capillary column methods of 20%, whereas our results for the percent recovery of 16 PAHs by Sep-Pak from synthetic water samples at pH 2 indicate a relative standard deviation of less than 10%. Percent recovery, as shown in Table 3, of all PAHs except naphthalene, is in the range of 80 to 94%.

Polycyclic Aromatic Hydrocarbons (PAH) in Welland River

The lower Welland River is the recipient of domestic effluents from the City of Welland and of liquid wastes from many industrial plants. Industries include steel plants, textile mills, abrasive plants, a large chemical plant and various support industries. All of these operations contribute to the load of effluents carried into the lower Welland River.

TABLE 2: Percent Recovery of PAHs from Synthetic Water Samples Without pH Adjustment

	% Recovery Liquid-Liquid Extraction		% Recovery Sep-Pak Extraction		
Nph	39	44	0	0	20
Acelene	62	33	28	51	60
Ace	60	21	46	65	74
Fl	70	29	0	78	72
Phe	80	83	0	106	-
An	79	55	65	89	79
Ft	78	14	81	105	43
Py	95	80	63	108	59
B(a)An	77	-	56	85	25
Chy	78	-	41	77	20
B(b)Ft	77	32	58	71	47
B(k)Ft	72	79	44	64	31
B(a)Py	69	73	46	70	37
diB(a,h)An	72	57	0	30	0
B(g,h,i)Per	72	56	23	34	19
In(1,2,3-cd)Py	76	59	18	67	31

Recovery of B(g,h,i)Per from synthetic water samples without pH adjustment:
Liquid-liquid extraction gave 66, 55 and 60% recovery.
Sep-Pak extraction gave 25, 63 and 55% recovery.
Same experiments with pH adjusted to 2:
% Recovery of B(g,h,i)Per by liquid-liquid extraction - 95.5% (average of 4 runs)
-by Sep-Pak extraction - 89.8% (average of 4 runs).

TABLE 3: Recovery of 16 PAHs from Synthetic Water Samples at pH 2

PAH	Sep-Pak Extraction Percent Recovery (average of 5 runs)	Relative Standard Deviation (%)
Nph	13.2	-
Acelene	82.9	9.7
Ace	79.7	5.6
Fl	83.5	6.1
Phe	87.5	7.7
An	86.8	7.6
Ft	91.3	7.0
Py	84.8	9.3
B(a)An	89.4	5.8
Chy	91.3	9.0
B(b)Ft	92.7	6.7
B(k)Ft	92.0	5.6
B(a)Py	92.5	6.2
diB(a,h)An	93.9	4.6
B(g,h,i)Per	93.8	5.9
In(12,2,3-cd)Py	94.4	6.3

FIGURE 1: Chromatogram of Supelco Standard (10 x diluted) Consisting of 16 PAHs.

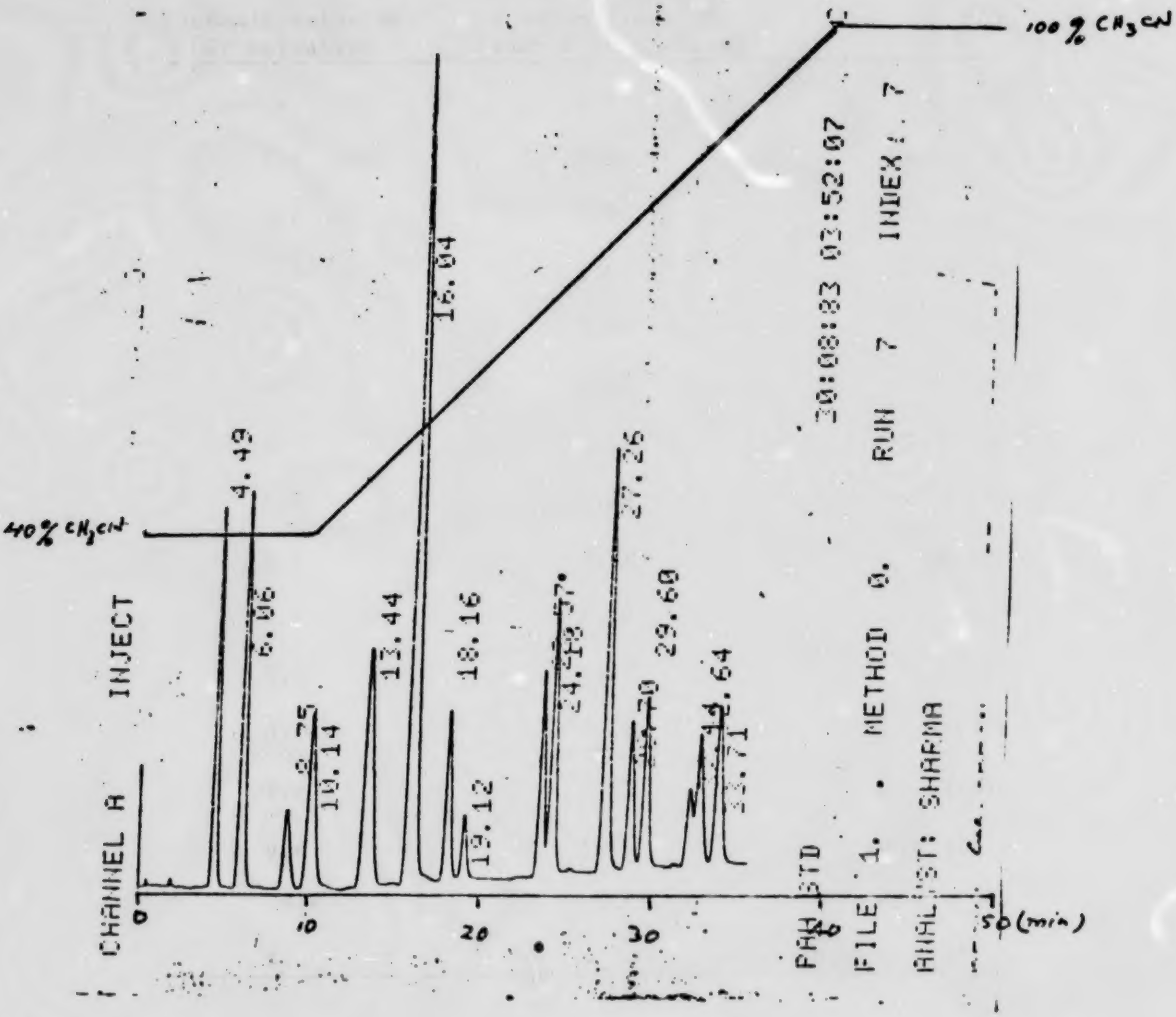
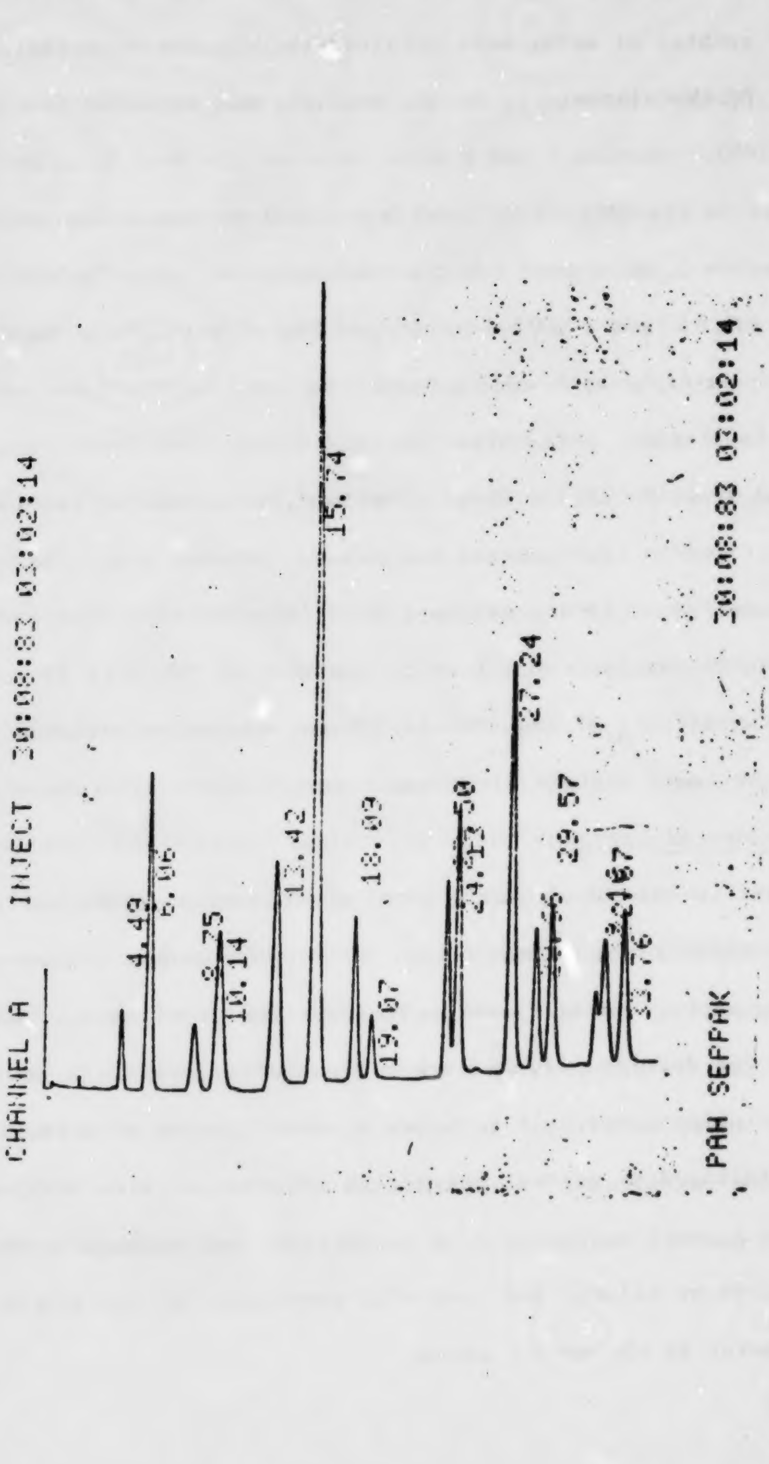


FIGURE 2: Chromatogram of 16 PAHs Extracted by Sep-Pak from Synthetic Water Sample



In order to determine the presence and concentration of PAH compounds in this river, samples of water were obtained through the cooperation of Professor M. Dickman, Brock University,, St. Catherines, who supplied four 1-litre samples on May 25, 1982. Samples 1 and 2 were taken at the West Main and Prince Charles steel bridges in the City of Welland (specified as downstream samples). Upstream samples 3 and 4 were taken at Wellandport, near Gladman's Park.

Steele and Dickman (35) have reported the effects of pollution of the lower Welland River on fish which were found to be considerably less numerous than the fish population living in the upper Welland River. Furthermore, in the lower river, in the vicinity of the City of Welland, about 48% of the carp-goldfish hybrids were found to have gonadal neoplasms. On ther other hand, no evidence of neoplasms was found in any of the 1,066 fish collected from the upper Welland River which drains agricultural land to the west of the City of Welland. Other evidence of the effects of industrial effluent wastes on selected aquatic plants and fish in the lower Welland River has been presented by Dickman et al. (36).

Analysis and Identification

Techniques in our laboratory for the quantitative determination of PAH pollutants in water samples employ High Performance Liquid Chromatography (HPLC) and spectrophotometric identification as described previously. The method is applicable to the determination of other organic pollutants in raw water and chlorinated drinking water. It involves a concentration step to reduce the sample size, followed by solvent extraction of organics with dichloromethane, evaporation to a small volume at room temperature and subsequent HPLC analysis, using reversed-phase columns and isocratic conditons, with mixtures of acetonitrile-water as the mobile phase.

Trace concentrations of PAHs can be separated into subgroups as follows, under isocratic conditions:

a) Two and three membered ring systems separated by 60/40 and 50/50 CH_3CN/H_2O

b) Four membered ring systems separated by 75/25 CH_3CN/H_2O

c) Five membered ring systems separated by 85/15 CH_3CN/H_2O

d) PAHs of higher molecular weight separated by 95/05 CH_3CN/H_2O

The PAH standards used in this study were obtained in as pure form as possible from various sources, including Aldrich, Sigma and Supelco. The Supelco standards contained 16 PAH compounds, shown in Table 1, listed as priority pollutants by the U.S. Environmental Protection Agency (EPA). These compounds were obtained as solutions in methanol/dichloromethane 50/50 (v/v) and were diluted further, 1 ml to 10 ml in acetonitrile prior to use as HPLC standards.

The Welland River water samples were passed through C_{18} Sep-Pak cartridges in order to remove the organic compounds. In the case of samples 1 and 2 (City of Welland, downstream samples), 1 litre of sample was passed through the C_{18} cartridge, whereas in the case of samples 3 and 4 (Wellandport, upstream samples), 500 ml of sample was passed through the C_{18} cartridge. The PAH compounds in each cartridge were removed by extraction with successive portions of dichloromethane. For each sample, the extract fractions were combined and dried by passage through a bed of anhydrous $MgSO_4$. The volume of each extract was reduced in a Kuderna-Danish evaporator to about 5 ml, and the remaining dichloromethane was removed by evaporation at room temperature under a stream of nitrogen. Then 0.5 ml of acetonitrile was added to the organic residue.

The solvent program for HPLC analysis, using various percentages of acetonitrile in water, included a reversed-phase column and ultraviolet detection at 254 nm. The PAH compounds of high molecular weight, containing 5 and 6 condensed aromatic ring systems are separated by solvents containing 85% and 95% acetonitrile in water. Lower concentrations of acenotnitrile in water must be employed to separate compounds consisting of 2 to 4 aromatic rings.

The HPLC separation of PAH from the Welland River samples are illustrated in Figure 3 for upstream samples 3 and 4, and in Figure 4 for the downstream samples 1 and 2, on the basis of µl injections of concentrated PAH extracts. The numbers on the various peaks in each chromatogram identify PAH compounds listed in the standards.

The concentrations, shown in Table 4, were determined by first evaluating the response factors (RF), defined as the ratio of the peak area to the amount of analyte standard injected. Thus, if analysis of a sample extract yields X ng injected of a given PAH (peak area times response factor), the concentration of this compounds in the original water samples is given by the formula

$$C = X / (rV) \ \text{ng/l}$$

where C = concentration, r is the ratio of the injection volume to the total extract volume and V is the volume in litres of the original water sample. The extraction efficiency for each PAH has been taken at 100%.

The results in Table 4 indicate that the concentrations of the eight listed PAH compounds are considerably higher in the water taken upstream at Wellandport than the concentrations in the water collected under the steel bridge at the City of Welland.

Carcinogenic and Mutagenic Activity

The carcinogenic and mutagenic activities of the eight PAH compounds found in the Welland River water samples are listed in Table 5. Dipple (37) has reviewed extensively the findings of many investigators' in vitro and in vivo studies of polynuclear aromatic carcinogens and the information in Table 5 on carcinogenic activity is based on data listed in this review.

The remarks regarding findings of mutagenic activity of the PAH (Table 5) are based on tests using the Ames assay as published by Salamone, Heddle and Katz (38). In general, the various results based on the Ames assay agree with the reported carcinogenic potency of these PAH.

The results of studies of some PAH concentration levels (ng/l) in several U.S. river waters are listed in Table 6 for purposes of comparison with values obtained in the Welland River samples (Table 4). The comparatively high values quoted by Basu and Saxena (39) were found in water collected in the Monogahela River, near Pittsburg, contaminated with coke oven effluent.

The analysis of the above water samples from the Welland River was undertaken prior to the study of the efficiency of Sep-Pak cartridges for the quantitative removal of PAHs in water. Since it has been revealed that requirements for quantitative recovery of dissolved PAH involves an activation procedure for the Sep-Pak and acidification of the water sample to pH 2, the above PAH concentrations in the Welland River samples must be accepted with reserve, as the data are unsupported by evidence of recovery efficiency.

TABLE 4: PAHs in Welland River Samples

	Up stream (ng/l)	Down stream (ng/l)
Chrysene	7.3	2.5
Benzo(e)pyrene	40	6.4
Perylene	21	5.0
Dibenz(a,c)anthracene	68	10
Benzo(a)pyrene	17	4.6
Dibenz(a,h)anthracene	72	24
Benzo(ghi)perylene	129	33
Indeno(1,2,3-cd)pyrene	2.0	0.5

TABLE 5: Carcinogenic and Mutagenic Activity of PAH in Welland River Samples

PAH Compound	Carcinogenic activity (Dipple)	Mutagenic activity (Salamone, Heddle & Katz)
Chrysene	disputed	slight
Benzo(e)pyrene	inactive	doubtful
Benzo(a)pyrene	high	high
Perylene	inactive	inactive
Dibenz(a,c)anthracene	disputed	mild
Dibenz(a,h)anthracene	moderate	mild
Benzo(ghi)perylene	moderate	mild
Indeno(1,2,3-cd)pyrene	moderate	-

TABLE 6: PAH Concentration Levels in Some U.S. River Waters (concentrations in ng/l)

PAH Compound	Crane et al. (40)	Organ et al.(41)	Basu and Saxena (39)
Chrysene	-	70	-
Benzo(a)pyrene	17	6	42
Benzo(ghi)perylene	19	16	34
Indeno(1,2,3-cd)pyrene	10	10	60

FIGURE 3.

SOLVENT: 85% ACETONITRILE

SAMPLE: UPSTREAM SAMPLE

FROM WELLAND RIVER

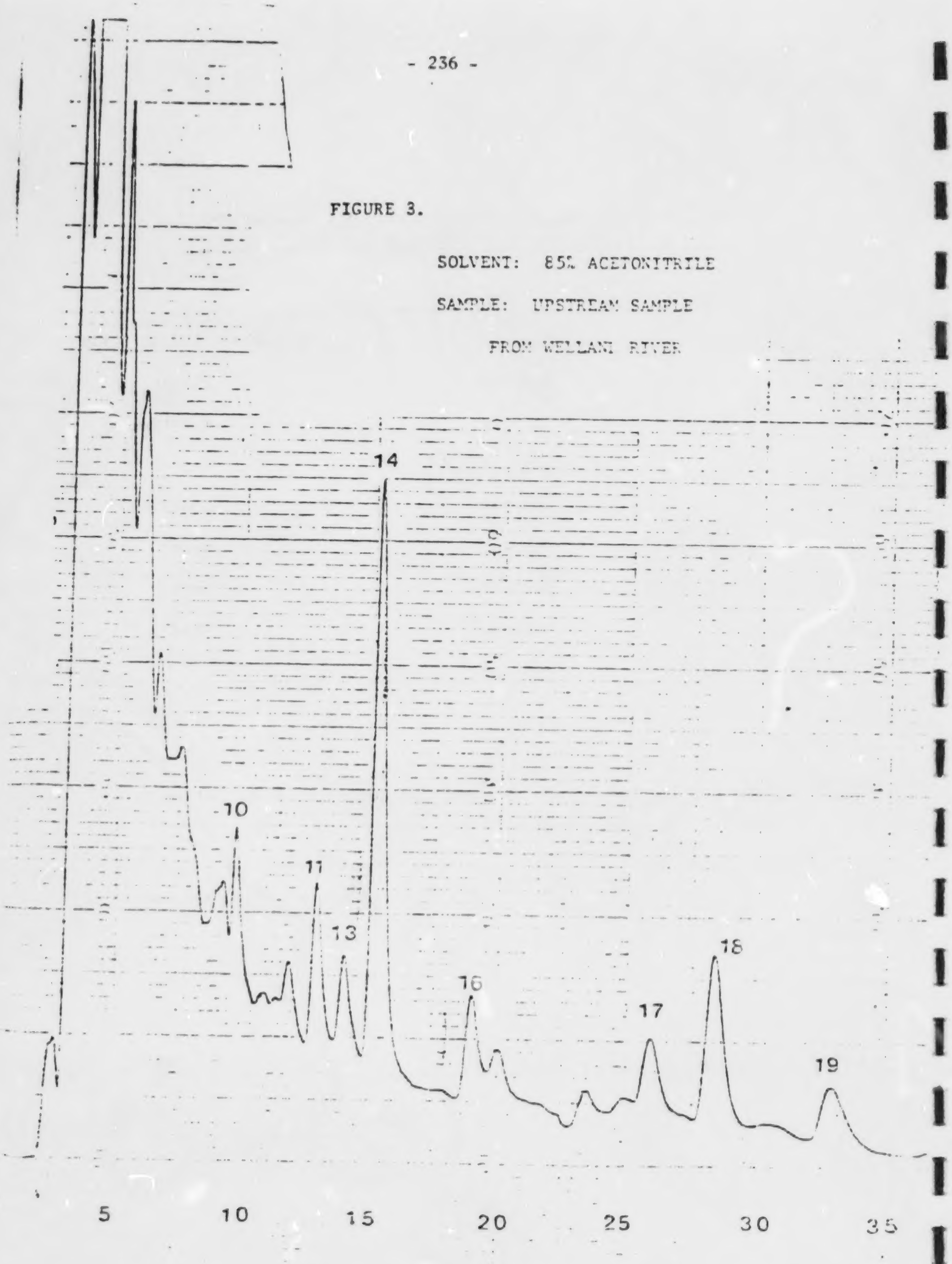
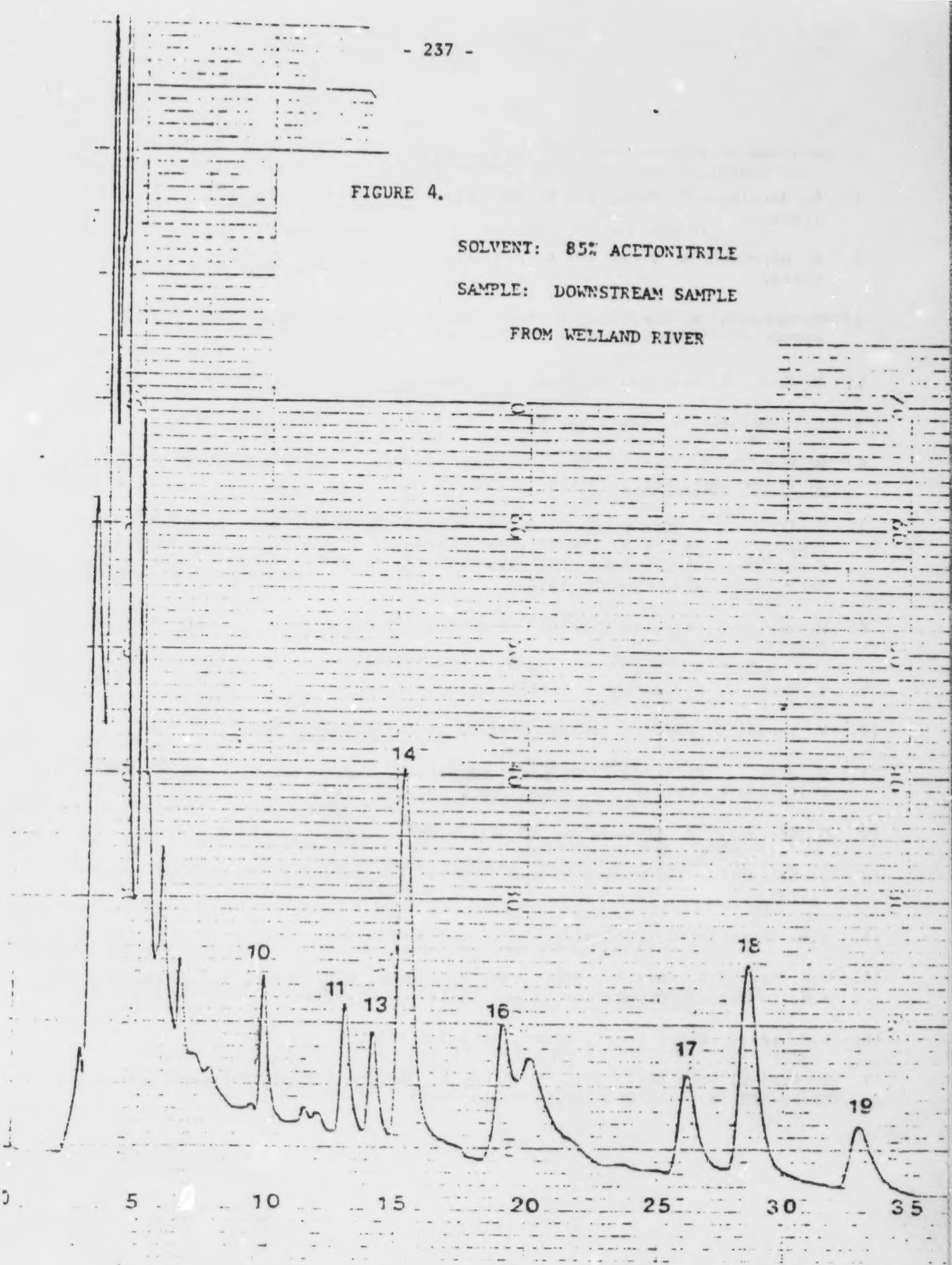


FIGURE 4.

SOLVENT: 85% ACETONITRILE

SAMPLE: DOWNSTREAM SAMPLE
FROM WELLAND RIVER



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II. BIOLOGICAL STUDIES OF ENVIRONMENTAL MUTAGENS, INHIBITORS AND PROMOTERS

Introduction

It has been estimated that 60% or more of the incidence of cancers in man and animals is due to the action of environmental chemicals or pollutants. Anthropogenic sources of carcinogenic and mutagenic chemicals include the products of incomplete combustion of fossil fuels, exhaust gases from motor vehicles, emissions from coke ovens and from many other industrial activities, such as oil refining, chemical manufacturing and metal processing. Polycyclic aromatic hydrocarbons (PAH) are a particularly important group of carcinogenic pollutants but there are many other classes of environmental substances that constitute potential carcinogenic or mutagenic hazards to health, including chlorinated and fluorinated hydrocarbons, alkylating agents, amines and nitrosamines, and other organic compounds and inorganic chemicals containing Be, Cd, Mn, Ni, Co, Pb and Cr.

Extensive reviews of the activity of many chemical carcinogens have been published by Heidelberger (1), Searle (2), the Millers (3), the U.S. National Academy of Sciences (4) and others (5,6,7). Apart from anthropogenic sources, a considerable number of carcinogens are products of natural origin and occur in plant species and micro-organisms, e.g., alkaloids, constituents of essential oils, aflatoxins. The anthropogenic chemicals, such as PAH, are prevalent contaminants of air, water and soil.

Various theories have been proposed to explain the mechanism of action of chemicals in terms of species and tissue susceptibility, biocehmical pathways for activation and detoxification and elucidation of the role of specific classes of compounds in human carcinogenesis. An extensive review of the chemistry and biological effects of PAH has been presented in the proceedings of a symposium edited by Bjorseth and Dennis (8). These compounds can bind covalently to cellular macromolecules such as DNA, RNA and protein. The amount of hydrocarbon bound is proportional to the concentration of macromolecule in the tissue, with most of the hydrocarbon being found by cellular protein, followed by RNA and DNA. Since PAH are metabolized by a microsomal mixed-function oxidase enzyme called aryl hydrocarbon hydroxylase (AHH), the resultant activation process may lead to the formation of an intermediate epoxide species which is a much more active carcinogen than the parent hydrocarbon. The epoxide can be converted into a glycol or diol by a second microsomal enzyme, epoxide hydrase, which may lead to detoxification and rapid removal of the reactive epoxide before interaction with a target site (9). However, in some cases the diols of PAH can undergo another cycle of metabolic activation and become even more carcinogenic, as diol epoxides, than the initial epoxide (10). The diol epoxide of benzo(a)pyrene (BaP) has been shown to be an active mutagen (11).

Aromatic amines and nitrosamines also require metabolic oxidation by hepatic microsomal mixed-function oxidases to become tumorigenic (9). Carcinogenic substances that do not require metabolic activation are biological alkylating agents, such as alkyl alkanes, epoxides, sulfonates, strained ring lactones, and nitrosamides. Inorganic carcinogenic chemicals containing certain toxic metals and metalloids, and asbestos, are also effective without metabolic activation.

A review of the etiology, mechanisms and prevention of cancer has been presented by Slaga (12). Chemical carcinogenesis is a multistep process as demonstrated by studies with experimental animals. In addition to the action of tumor initiators and promoters, other factors include the effect of the presence of other carcinogens acting to produce additive, synergistic or inhibitory effects. Agents are also known that can act to inhibit the process by modifying metabolic pathways to counteract or detoxify the carcinogen. The DNA repair mechanism is an important determining factor in the cancer initiation of a cell by a chemical carcinogen or mutagen. Interference or inhibition of the DNA excision repair system may lead to the irreversible carcinogenic process.

Some of the known cancer promoters include croton oil and its phorbol esters, fatty acids and esters, tobacco smoke condensate, phenols, DDT and PCB. Various chemical agents have been studied that are potential inhibitors of both the initiation and promotion phases of carcinogenesis. These substances have the capacity to inhibit tumor initiation or promotion by one or more of the following pathways (13,14): (a) alteration of the metabolism of the carcinogen by microsomal enzymes, leading to decreased activation and/or increased detoxification, (b) scavenging of active carcinogens to prevent their approach to critical target sites in the cells, (c) inhibition of promotion or progression by altering the state of differentiation, (d) inhibition of promoter-induced cellular differentiation, (e) prevention of gene activation by promoters. Inhibitors that have been investigated include flavones, proteases, retinoids, anti-inflammatory steroids, and a number of antioxidants. Cruciferous plants and citrus fruits contain certain chemicals that act as inhibitors and inducers of the mixed-function microsomal oxidase system and result in enhanced detoxification of carcinogens (15).

Within recent years, the concern regarding the continued increase in the numbers of chemical carcinogens in the environment has led to greatly increased efforts to survey, identify and study the chemistry and biological effects of potential carcinogens. However, conventional tests with animals, especially for unknown or weak carcinogenic activity, require costly studies for long periods. Current standards for testing carcinogens in mice involve a cost of at least $150,000 for a single chemical. Such cost and the two-year time period required for each test render the conventional methods completely unsuitable for experiments involving a large number of chemicals and combinations of chemicals. Typical human exposure to air pollutants involves not merely single compounds but rather complex mixtures of large numbers of PAH and other chemicals.

Much more rapid and economical testing methods have been developed in recent years. These new methods for detecting carcinogens depend upon the ability of potential carcinogens to produce mutations or genetic damage in bacterial and mammalian cells. Both in vitro and in vivo biological assays are now available to detect agents that can produce mutations.

The following comparatively rapid and sensitive assays for mutagenic activity of chemicals have been employed in this study.

1. The Ames Salmonella typhimurium assay

This assay, developed by Ames and his co-workers (16,17), involves the use of histidine auxotrophs of the above bacteria, with strains TA1535 and TA100 each bearing a substitution-deletion mutation or TA1538, TA98 and TA1537 each containing a frame shift mutation. Results by McCann et al. (17) of 300 chemicals containing all known human carcinogens, except the heavy metals, have indicated that on the above strains 85% of the carcinogens are also mutagens.

2. In vivo mammalian assays

(a) A micronucleus assay, developed by Heddle (18), Schmidt (29) and Salamone et al. (23) detects damage to the chromosomes of the rapidly dividing erythroblastic cells in mice bone marrow. The test is based on the phenomenon that chromosome fragments which lack spindle attachment sites or centromeres are often left in the cytoplasm of dividing cells where they persist as micronuclei. Such structures are readily recognized and can be scored rapidly compared to the classical methods of metaphase chromosome analysis.

(b) The sperm abnormality assay detects point mutations leading to abnormal sperm head morphology as a result of exposure of male mice to irradiation and chemicals that are mutagens, teratogens and carcinogens (19,20,21).

Earlier studies in the field, published by our research group, have dealt with the mutagenic activity of 30 PAH and oxides in airborne particulates (22), development of an improved micronucleus test (23), mutagenicity of paired chemical mixtures (24), the mutagenic activity of 41 compounds on the in vivo micronucleus assay, as part of an international collaborative program (25), mutagenic activity of PAH and other environmental pollutants (26), the complexities of risk estimates, metabolic activation and chemical mixtures (27) and the effect of 5,6-benzoflavone on the mutagenicity of PAH (28).

Various chemicals which have been used in the above tests are listed below with their sources. Methods of operation have been described in the individual assays.

TABLE 1

Chemicals	Source	Mutagen/inhibitor/promotor
Dimethylbenz(a)anthracene (DMBA)	Eastman Kodak Co.	Promutagen
Benzo(a)pyrene (BaP)	Aldrich Chemical Co.	Promutagen
Cyclophosphamide (CP)	Aldrich Chemical Co.	Promutagen
Mitomycin C (MMC)	Boehringer Mannheim	Direct acting mutagen
Butylated hydroxyanisole (BHA)	Sigma Chemical Co	Possible inhibitor
α-naphthoflavone	Sigma Chemical Co.	Possible inhibitor
β-naphthoflavone	Sigma Chemical Co.	Possible inhibitor
Caffeic acid	Fluka Co.	Possible inhibitor
Ascorbic acid	BDH Chemical Co.	Possible inhibitor
Disulfiram	Sigma Chemical Co.	Possible inhitibor
α-tocopherol	Hoffman La Roche	Possible inhibitor
β-sitosterol	Sigma Chemical Co.	Possible inhibitor
Ethoxyquin	Aldrich Chemical Co.	Possible inhibitor
Corn oil	Mazola Brand/retail store	Possible inhibitor
Sunflower oil	Unico Brand/retail store	Possible inhibitor
Metyrapone	Aldrich Chemical Co.	Possible inhibitor
Arochlor	Sigma Chemical Co.	Promoter
Phenobarbital	Sigma Chemical Co.	Promoter
Hexachlorobenzene	Aldrich Chemical Co.	Promoter
Hexachlorocyclohexane	Aldrich Chemical Co.	Promoter

I. Bone Marrow Micronucleus Assay

Virgin B6C3 female mice (Charles River Canada Inc.) were 8-9 weeks of age when used for in vivo bone marrow micronucleus assay experiments. The mice ranged from 20-22g in weight and they were maintained under normal animal room conditions before and during experimental periods. All mice were fed with Purina Laboratory Rodent Chow and watered ad libitum. Five animals were housed per cage (cage dimensions, 19cm W x 29cm L x 12 cm H) and 5 animals were used per each treatment. The in vivo bone marrow micronucleus assay as described by Schmidt (29) and Salamone et al. (23) has been employed in the present studies. $LD_{50/7}$ was determined for the chemicals before being tested (Weil (30)).

The mice were injected intraperitoneally (i.p.) with the chemical dissolved in appropriate solvent and samples of bone marrow taken at various intervals starting from 24 hr after the treatment with test compounds. Micronucleated polychromatic erythrocytes (MNPCE) per 500 PCE were scored in each sample. Control animals were treated with solvent only and tested for the presence of micronuclei as above. Red blood cells (RBC) have also been counted per 100 PCE. The ratio of RBC/PCE gives the approximate estimate of toxicity of the test chemical.

In general, dimethyl sulfoxide (DMSO) has been used as a solvent. However, distilled water, physiological saline, and corn oil also have been used as appropriate solvents in some cases.

Potential inhibitors were injected 48 hr and 24 hr before treating the mice with promutagen or direct acting mutagen. By giving two injections 24 hr apart, one can give double the amount of chemical to the same mouse. So by injecting the presumed inhibitor 48 hr before injecting the mutagen, this probably facilitates building up of the aryl hydrocarbon hydroxylase enzyme system so that this enzyme complex can detoxify the effect of the promutagen. The mice were treated with the potential inhibitors listed in Table 1.

Some of the inhibitors were mixed in powdered food and the experimental mice were kept on that diet for at least 7 days before treating with the mutagen. Butylated hydroxyanisole (BHA), caffeic acid, disulfiram, ethoxyquin were the inhibitors fed in this way to the mice.

Potential Inhibitors of Mutagens - In Vivo Bone Marrow Micronucleus Assay

1. Butylated Hydroxyanisole (BHA)

Experimental mice were fed with powdered food containing BHA (5g/Kg of food) for one week before administering mutagens and continued feeding until the end of the experiment. Then those mice were injected i.p. either eith DMBA, BaP, CP or MMC. Bone marrow samples were collected at various intervals up to 96 hr starting from 24 hr after administering the mutagen. Our results showed that at 56 hr sampling time there was about 133% increase in the mean number of micronucleated polychromatic erythrocytes in BHA + DMBA treatment over DMBA treatment alone (Fig. 1). So, BHA showed a large promotory effect in the presence of DMBA. Our result was quite contradictory to the results of Wattenberg (14,15) where he indicated that BHA had inhibited the carcinogen-induced neoplasia either in lung or forestomach or both in mice.

Neither promotory nor inhibitory effect was noticed with BHA + BaP, BHA + CP or BHA + MMC.

2. 5,6 Benzoflavone or β-naphthoflavone

Mice wre pretreated i.p. with β-naphthoflavone (80mg/Kg in DMSO) for two days consecutively before treating with either DMBA (25mg/Kg in DMSO) or BaP (186mg/Kg in DMSO). In another set β-naphthoflavone, BaP and DMBA were dissolved in corn oil. In the former case, β-naphthoflavone dissolved in DMSO showed enhanced activity of DMBA instead of the expected reduction. However, in the latter case, where corn oil was used as a solvent for β-naphthoflavone as well as DMBA, the number of micronucleated PCE was reduced by 50%. Therefore, the actual inhibition was suspected to be due to corn oil.

β-Naphthoflavone in DMSO acted as an inhibitor by reducing the number of micronucleated PCE by 60% in the experiments where BaP was used as the promutagen. But, 100% inhibitory effect was noticed where β-naphthoflavone and BaP were dissolved in corn oil. Here also, corn oil could have actually enhanced the inhibition.

3. Alpha Naphthoflavone (7,8-benzoflavone)

In pulmonary adenoma test system of the mouse, flavone inducers inhibited lung tumor formation resulting from oral administration of DMBA or BaP (14,15). In other studies (5), it was reported that 7,8-benzoflavone inhibits hydrocarbon metabolism and hydrocarbon toxicity in hamster cell homogenates and rat liver microsomes only when these activities have been induced by prior treatments with benz(a)anthracene and 3-methylcholanthrene respectively. It has also been observed that 7,8-benzoflavone acted as a potent inhibitor of microsomal mixed function oxidase activity resulting ultimately in inhibition of epidermal neoplasia caused by DMBA (7,13).

The above information substantiates the inhibitory action of 7,8-benzoflavone in various types of cancer. Consequently we examined whether 7,8-benzoflavone would inhibit the formation of chromosomal breaks in mouse bone marrow tissue induced by DMBA or BaP.

Mice were injected, i.p., with 7,8-benzoflavone (80mg/Kg in DMSO) at 48 and 24 hr (2 injections per mouse) before treating with either DMBA (25mg/Kg in DMSO) or BaP (150mg/Kg in DMSO). Bone marrow samples were taken at prescribed times (24, 48, 72 and 96 hr after treatment with either DMBA or BaP).

Bone marrow cells from mice treated with 7,8-benzoflavone alone showed less than one micronucleated PCE per 500 PCE on average, as found also with DMSO. Maximum number of micronucleated PCE observed on average was 8.6 at 48 hr with DMBA and 11.6 at 72 hr with BaP. Considerable reduction in the average number of micronucleated PCE in the above treatments was found when the mice were pretreated with 7,8-benzoflavone. In other words, about 90% inhibition in case of DMBA and 75% in case of BaP was caused by 7,8-benzoflavone. Detailed results have been published elsewhere (Raj and Katz (31)).

4. Caffeic Acid

Caffeic acid (3,4-dihydroxycinnamic acid) is present in the free state only in small quantities in foods of vegetable origin but constitutes a significant portion in polymeric form of the lignin fibres that hold wood plant cells together. Caffeic acid is present in potatoes, sweet potatoes, apples, pears, carrots, cacao, coffee, wheat and many other plants. It occurs in plants only in conjugated forms with chlorogenic acid.

The experimental mice were fed for one week and throughout the experimental period with powdered food mixed with caffeic acid (2%). The following treatments were included in the experimental protocol: 1) control (untreated), 2) DMSO (solvent control), 3) caffeic acid control, 4) caffeic acid + DMSO, 5) DMBA in DMSO, 6) caffeic acid + DMBA. Bone marrow samples were collected at 24, 48, 60, 72 and 96 hr after treatment with DMBA. The samples collected at 60 hr from DMBA treated mice showed the maximum number of micronucleated PCE (about

14/500 PCE). At the same sampling time the bone marrow samples from the mice fed with caffeic acid showed about 7 micronucleated PCE/500 PCE; i.e., the inhibitory effect of caffeic acid is about 50% at 60 hr (Fig. 2). A detailed report has been published elsewhere (Raj et al. (32)).

5. Ascorbic Acid

Ascorbic acid (Vitamin C) is widely distributed in the plant and animal kingdom. Good sources are citrus fruits, hip berries, acerola, fresh tea leaves, etc. Aside form its use as a vitamin, ascorbic acid or some derivatives are employed as antioxidants in foodstuffs, to prevent rancidity, to prevent the browning of cut apples and other fruit, and in meat curing.

Effect of ascorbic acid was tested against DMBA, BaP, CP and MMC induced chromosomal breaks in mice using bone marrow micronucleus assay. Ascorbic acid, dissolved in physiological saline, was injected to experimental mice one day before mutagen treatment. The dose of ascorbic acid was 520mg/Kg (80% LD_{50}). Next day the mice were injected with either DMBA (25mg/Kg), BaP (186mg/Kg), CP (43mg/Kg), or MMC (1mg/Kg). Bone marrow samples were collected at 24, 48 and 72. hr.

Our results indicated that ascorbic acid itself produced about 2.1 micronucleated PCE/500 PCE at 48 hr. Inhibitory effect of ascorbic acid was seen both in DMBA and BaP treated mice. The inhibition in DMBA treated mice was about 40% at 48 hr and 30% at 24 hr; whereas in BaP treated mice the inhibition was about 66% at 48 hr and 50% at 72 hr. The situation is reverse in CP and MMC treatments. Instead of inhibition, promotory effect was noticed especially at 48 hr when ascorbic acid was used along with the mutagens.

From these results one could say that ascorbic acid acts as an inhibitor against certain mutagens (DMBA and BaP) and as a promoter in combination with CP or direct acting mutagen MMC.

6. Disulfiram

Disulfiram (tetraethylthiuram disulfide) was first produced in 1881 and was used in the rubber industry to accelerate the vulcanization of rubber. Disulfiram was introduced in the 1930's into the medical world as a scabiescide. It is an off-white, odorless, crystalline powder and was found to be toxic to lower forms of life utilizing copper-containing respiratory enzymes. Intestinal worms were especially sensitive to disulfiram and hence, this was introduced as a vermicide. Disulfiram was proposed for the prophylaxis of chronic alcoholism.

Azomethane is a carcinogen and the oxymetabolite of azomethane produces neoplasia (Fiola et al., 33) and disulfiram offers raido-protection from the oxymetabolite neoplasia, indirectly inhibiting, the metabolism, of azomethane.

Lang et al. (34) demonstrated that disulfiram and its major metabolite, diethyldithiocarbamic acid, diminished the inductive effect of phenobarbital on cytochrome P-450 content and p-nitro-anisole-O-demethylation, while both compounds showed additive effect in inducing NADPH-dependent cytochrome reductase. These studies indicated that disulfiram acted as an inhibitor against certain promoters (e.g., phenobarbital). Therefore, we conducted experiments using disulfiram to see whether there would be any inhibitory action against DMBA, BaP, CP and MMC-induced chromosomal breaks, using the bone marrow micronucleus test.

Experimental mice were fed for 10 days on a diet containing 1% disulfiram and on the 11th day the mice were injected i.p. with mutagens. Bone marrow samples were collected at regular intervals. In the case of DMBA-induced chromosomal breaks, an inhibition of about 80% was noticed by disulfiram in the 48 hr sample. In the case of the other mutagens, no inhibitory activity was caused by disulfiram.

7. Metyrapone

Metyrapone (Metopirone, or 2-methyl-1,2-di-3-pyridyl-1-propanone) is used as a diagnostic tool for the determination of residual pituitary function in patients with hypopituritarism (Sprunt et al., 35; Meikel et al., 36). It has also been shown to inhibit a number of hepatic cytochrome P-450 dependent monooxygenations such as aromatic ring hydroxylation, and O- and N-dealkylation (Netter et al., 37; Kohl et al., 38; Hildebrandt, 39). This encouraged us to study the possible inhibitory effect of metyrapone against formation of chromosomal breaks caused by different mutagens.

Mice were injected with metyrapone (100mg/Kg) 48 and 24 hr before injecting either with DMBA (25mg/Kg) in DMSO, or BaP (150mg/Kg) in DMSO and the bone marrow samples were obtained at various intervals. Metyrapone, by itself, produced only about 1 MNPCE/500 PCE at all sampling points. DMBA treated mice showed, on average, a peak number of 7.0 MNPCE/500 PCE at 48 hr and metyrapone pretreated mice that received DMBA showed about an average of 7.4 MNPCE/500 PCE at the same sampling time. On the contrary, BaP-treated mice showed about 8 MNPCE/500 PCE at 48 hr and pretreatment with metyrapone followed by treatment with BaP showed about 3.6 MNCPCE/500 PCE which indicates about 56% inhibition against BaP.

8. Ethoxyquin

The effect of ethoxyquin, a non phenolic antioxidant, was studied against DMBA, BaP, CP or MMC treated mice for *in vivo* bone marrow chromosomal breaks. Experimental mice were fed with a powdered diet mixed with ethoxyquin (10g/Kg of food) for one week before injecting the mice with the above mutagens and continued till the end of experiment. Mutagens DMBA (25mg/Kg in DMSO), BaP (186mg/Kg in DMSO), CP (43mg/Kg in saline) and MMC (1mg/Kg in saline) were injected and the bone marrow samples were obtained at regular intervals starting from 24 hr after mutagen treatment. Overall inhibition was observed at several time points in ethoxyquin fed mice treated with DMBA. However, 54% of inhibition was noticed particularly at the 24 hr sample. In the case of the CP treated mice, about 70% of inhibition was caused by ethoxyquin in the 24 hr sample (Fig. 3). In MMC treatment up to 40 hr sampling times (i.e., 24 and 40 hr) about 90% of inhibition was observed (Fig. 4). BaP treated mice did not react positively with ethoxyquin in showing inhibition. All the results pertaining to the inhibitors (2 to 8) are presented in Table 2 in consolidated form.

TABLE 2: Effects of Potential Chemical Inhibitors Against Mutagens in Bone Marrow Micronucleus Assay with Mice

Chemical Mutagen	Solvent for Mutagen	Inhibitor	Application	Extent of Inhibition % Bone Marrow Micronucleus
DMBA	Corn oil	5,6-benzoflavone	Corn oil, i.p.	50
BaP	DMSO	5,6-benzoflavone	DMSO, i.p.	66
BaP	Corn oil	5,6-benzoflavone	Corn oil, i.p.	100
DMBA	DMSO	Caffeic acid	2% in feed	50
DMBA	DMSO	7,8-benzoflavone	DMSO, i.p.	90
BaP	DMSO	7,8-benzoflavone	DMSO, i.p.	75
DMBA	DMSO	Ascorbic acid	Saline, i.p.	40
BaP	DMSO	Ascorbic acid	Saline, i.p.	66
DMBA	DMSO	Disulfiram	1% in feed,10 days	80
DMBA	DMSO	Metyrapone	i.p. pretreated	none
BAP	DMSO	Metyrapone	48 and 24 hr	56
DMBA	DMSO	Ethoxyquin	In feed, 1 week	54
CP	Saline	Ethoxyquin	In feed, 1 week	70
MMC	Saline	Ethoxyquin	In feed, 1 week	90

DMBA = 7,12-diemthylbenz(a)anthracene
BaP = Benzo(a)pyrene
CP = Cyclophosphamide
MMC = Mitomycin C

9. Corn Oil and Its Minor Constituents, and Sunflower Oil

The possible inhibitory effect of corn oil against polynuclear aromatic hydrocarbon-induced chromosomal breaks was suspected when studying the effect of beta-naphthoflavone (BNF) against DMBA. BNF dissolved in corn oil showed much more inhibiton than BNF in DMSO. In other treatments wherever corn oil was present, the inhibition was much more pronounced. Therefore, the effect of corn oil was studied sytematically, as follows.

8-10 weeks old female B6C3F1 hybrid mice were injected i.p. with corn oil (0.4ml/20g mouse) for two days consecutively, i.e., 48 and 24 hr before injecting them with promutagen DMBA. The following is a list of experimental treatments:

1) DMBA was dissolved in corn oil and injected in the mice which were pretreated with corn oil.
2) DMBA was dissolved in corn oil and injected in the mice which were pretreated with DMSO.
3) DMBA was dissolved in DMSO and injected in the mice which were pretreated with corn oil.
4) DMBA was dissolved with DMSO and injected in the mice which were pretreated with DMSO.
5) Mice were injected with DMBA dissolved in corn oil (without any pretreatment).
6) Mice were injected with DMBA dissolved in DMSO (without any pretreatment).

The bone marrow samples were collected at 24, 48, 72 and 96 hours after the last injection in the respective treatments. Reduction in number of MNPCE were observed at all points wherever corn oil was used as solvent. More than 50% inhibition was noticed at 72 hr sampling time. Pretreatment with corn oilshowed inhibition over untreated mice.

The minor constituents of corn oil were tested against DMBA-induced chromosomal breaks. Sunflower oil was included in our studies. Minor constituents of corn oil include gallates, α-tocopherol and β-sitosterol. Mode of treatment and the results are consolidated in Table 3.

TABLE 3: Corn Oil and Its Minor Constituents as Inhibitors Chromosomal Breakage Induced by DMBA In Vivo Bone Marrow Micronucleus Assay with Mice

Inhibitor	Pretreatment	Solvent for DMBA	MNPCE Sampling time, hr.	% Inhibition
Corn oil	-	DMSO versus Corn oil	72	>50
Corn oil	(0.4ml/20g mouse) i.p. for 2 days*	DMSO	72	35
Corn oil	i.p. for 2 days	Corn oil	48	70
Corn oil	i.p. for 2 days	Corn oil versus DMSO	72	60
Propyl gallate	(217mg/Kg)	DMSO	48 and 72	none
γ- Tocopherol	1% in food for 4 days	DMSO	48	ca, 60
β- Sitosterol	(150mg/Kg) i.p. in 50% ethanol	DMSO	48	60
Sunflower oil	(0.4ml/20g mouse) i.p. for 2 days	DMSO	48	75

*Interperitoneal injections of inhibitor at 48 hr and 24 hr prior to DMBA treatment.

It is evident that mainly two natural ingredients, α-tocopherol and β-sitosterol, are the principal ingredients in corn oil that contributed to the inhibitory action against DMBA-induced chromosomal breaks. Not only corn oil, but also sunflower oil has the capacity to protect against clastogenic action caused by DMBA. Corn oil has 86% unsaturated fatty acids and 13% saturated fatty acids, whereas Unico Sunflower Oil has 65% unsaturated fatty acids and 12% saturated fatty acids and traces of beta carotene. Unsaturated fatty acids are recognized as essential dietary elements, and helpful to human health. Blood cholesterol levels are lower when unsaturated vegetable oils, instead of saturated fats, are used in the diet.

There are several reports dealing with the pros and cons regarding the effect of corn oil. Diets which provide a source of polunsaturated fatty acid enhance the in vivo metabolism of xenobiotics (Michalik et al., 40; Chadwick et al., 41) and result in alterations of pharmacologic responses to drugs (40; Wade et al., 42,43). The increased concentrations of cytochrome P-450 and other key components of the hepatic mixed function oxidase systems appear to be responsible for the enhanced in vitro and in vivo metabolism of drugs and other xenobiotics by animals fed these polyunsaturated fat diets (Chen et al., 44; Lam and Wade, 45,46; Lambert and Wills, 47). Corn oil and sunflower oil pretreament in our experiments must have caused induction of the mixed function oxidase system to the extent that it resulted in metabolism of DMBA as indicated in Table 3.

II. Abnormal Spermhead Assay

B6C3F1 male mice (Charles River Canada Inc.) were 11 to 14 weeks of age when used for sperm abnormality assay. The mice ranged from 24-27g in weight and they were maintained under normal animal room conditions before and during experimental periods. All mice were fed with Purina Laboratory Rodent Chow and watered ad libitum. All the chemicals were injected i.p. and the sperm cell samples were collected from Cauda epididymides on the 35th day after the last injection and sperm smears were prepared according to Wyrobek and Bruce (20). At least 500 sperm cells were scored at a magnification of 400x and a number of abnormal sperm cells were counted, using the "normal and abnormal murine sperm chart" of Wyrobek et al. (19,21). Sperm abnormalities might permit a simple quantative assay for damage to the genetic material of the male germ line.

10. Ferulic Acid - Inhibiton of Mutagen-Induced Germ Cell Abnormality

Ferulic acid and caffeic acid have been shown to diminish the incidence of forestomach tumors of mice treated with BaP (Wattenberg et al., 48). Recently, Wood et al. (49) reported the inhibitory effect of naturally occurring plant phenols, including ferulic acid, against the mutgenicity of bay-region diol epoxides of polycyclic aromatic hydrocarbons using the Ames Salmonella assay and Chinese hamster cells in vitro. Newmark and Mergens (50) found that caffeic and ferulic acids were particularly effective in reactions with nitrite and thus blocking the formation of carcinogenic N-nitroso compounds. Since ferulic acid is widely distributed among fruits and vegetables that are regularly consumed by humans, a study was made of the possible inhibitory effect of ferulic acid against DMBA-, BaP-, CP- and MMC-induced abnormalities in germ cells.

The male mice received pretreatment by being fed on a powdered diet containing 2% ferulic acid for one week before receiving the appropriate mutagen. On the 35th day after the last injection, sperm samples were collected and the cells analyzed for abnormal sperm cells.

The following doses of mutagens were given to mice i.p.:

DMBA:	20mg/Kg	2 injections
BaP:	150mg/Kg	1 injection
CP:	45mg/Kg	1 injection
MMC:	0.8mg/Kg	4 injections.

The results are summarized in Table 4.

TABLE 4: Results from Action of Ferulic Acid on Mutagen-Induced Germ Cell Abnormality

Treatment	No. of mice treated	No. of mice surviving till 35th day	Mean no. of abnormal sperm/ 500 sperm±S.E.
Ferulic Acid (FA)	5	5	11.0 ± 3.4
DMBA	5	1	11.7
BaP	5	0	Toxic
CP	6	6	8.7 ± 2.6
MMC	5	5	7.5 ± 0.8
DMBA+FA	10	5	13.2 ± 1.8
BaP+FA	10	3	16.5 ± 3
CP+FA	10	8	9.9 ± 1.5
MMC+FA	10	7	17.2 ± 4.3

The results shown in Table 4 indicate that ferulic acid in the feed apparently produced a relatively high number of abnormal germ cells, comparable to that produced by DMBA alone and higher than that produced by CP and MMC, acting alone. BaP was toxic to mice at the dose employed. It is presumed that if FA had no inhibiting effect on the above mutagens, the mean number of abnormal sperms would in a given combination be represented by the sum of FA plus mutagen. However, DMBA + FA yielded 13.2 instead of the expected total of 22.7; CP + FA, 9.9 instead of 19.7 and MMC + FA, 17.2 instead of 18.5. Therefore, it can be tentatively concluded that ferulic acid has an inhibitory effect on abnormal germ cells induced by DMBA and CP, but the results with BaP are obscured by its toxicity to mice at the dose used of 150mg/Kg.

11. α- and β-Naphthoflavones - Inhibitors of Mutagen-Induced Germ Cell Abnormality

Experimental mice were injected i.p. either with α-NF or β-NF (both 80mg/Kg) for two days consecutively at 24 hr intervals before injecting DMBA (20mg/Kg) for the next two days successively. Five mice were used in each treatment, except in the α-NF treatment where only 4 mice were used. Sperm cell samples were collected from cauda epididymides on the 35th day after the last injection and sperm smears were prepared.

The results are summarized in Table 5. The survival rate of treated mice was good except in two treatments. The survival was 3 out of 5 in DMBA alone, and 2 out of 5 in β-NF + DMBA. The range of abnormal sperm cells was more or less the same in untreated controls, solvent controls α-NF and β-NF. Number of abnormal sperm cells in DMBA treatment was high, ranging between 74-138 in different mice with an average of 108±18.6. Considerable reduction in number of abnormal cells was noticed both in α-NF (83%) and β-NF (60%) pretreated mice.

TABLE 5: Inhibitory Effect of α-Naphthoflavone (α-NF) and β-Naphthoflavone (β-NF) on DMBA-Induced Abnormal Sperm Cells.

Treatmen	No. Mice Used	No. Mice Survived*	Range of Abnormal Sperm Cells/500 Sperm Cells	Mean±S.E.
1. Untreated	5	5	6-14	9.2 ± 1.6
2. DMSO (solvent control)	5	5	7-20	13.6 ± 2.5
3. α-NF (2x)**	4	4	8-16	11.5 ± 1.7
4. β-NF (2x)	5	5	6- 12	8.8 ± 1.0
5. DMBA (2x)	5	3	74-138	108 ±18.6
6. α-NF(2x) + DMBA (2x)	5	5	7- 26	18.8 ± 3.4
7. β-NF(2x) + DMBA(2x)	5	2	40- 46	43.0 ± 3.0

* Mice survived til 35th day after last injection.
** 2x indicate 2 injections, 24 hr apart in two days.

Studies with flavones, such as α- and β-NF have shown that animals can be protected from some of the deleterious health hazards caused by PAHs. It was observed that α-NF acted as a potent inducer of increased mixed-function oxidase activity, resulting ultimately in inhibition of epidermal neoplasia caused by DMBA (Gelboin and Ts'O, 5; Slaga, 7; Wattenberg, 51). The clastogenic acitivty of DMBA was found to be inhibited by α-NF (Raj and Katz, 31) as well as by β-NF (Salamone et al., 28). Furthermore, pulmonary and skin tumors in mice and mammary tumors in rats, resulting from exposure to DMBA, were shown to be inhibited by prior treatment with benzoflavones (13).

PAIRWISE COMBINATIONS OF MUTAGENS IN BONE MARROW MICRONUCLEUS ASSAY

People are exposed to an environment which contains a mixture of pollutants, especially in the urban industrial air where contaminants include polynuclear aromatic hydrocarbons (PAH). The effect of paired mixtures of DMBA and BaP were studied, using the in vivo bone marrow micronucleus assay.

Preliminary experiments have been conducted with a combination of DMBA + BaP in treatment with doses of 15mg/Kg for DMBA and 95mg/Kg for BaP. The results are presesented in Table 6. These treatments at half the usual dosage produced less than the average maximum MNPCE of a single PAH exposure. Therefore, we repeated the experiments with normal doses, i.e., DMBA (30mg/Kg), BaP (186mg/Kg).

In 48 hr samples, the average number of MNPCE/500 PCE were 7.2±1.1 (DMBA), 7.4±1.6 (BaP) and 4.4±1.2 (DMBA + BaP), indicating an antognistic effect instead of an additive effect. These results confirm our earlier results.

TABLE 6: Pairwise Combination Experiments with Mutagens Bone Marrow Micronucleus Assays with Mice

Mutagen	Dose	Max. Average MNPCE/500 PCE	Sampling time, hr.	Remarks
DMBA	15m/gKg	5.8	48	
BaP	95mg/Kg	8.0	60	
DMBA + BaP		3.6	48	Non-additive
DMBA + BaP		6.2	60	(overlapping standard error bars)

Other sets of experiments were also conducted using paired mixtures. One set of mice was treated with a mixture of DMBA (30mg/Kg) and MMC (1mg/Kg), and another set was treated with BaP (186mg/Kg) and CP (45mg/Kg). In 48 hr samples, the average numbers of MNPCE/500 PCE were as follows: DMBA (7.2±1.1), MMC (7.2±1.8), CP (8.0±2.0), DMBA + MMC (23.5±1.3), BaP + CP (21.0±1.0). The additive effect was 163% in the combinaiton of DMBA + MMC and 136% in the combination of BaP + CP. In the DMBA + MMC combination, out of 5 mice only 2 mice survived. Each mouse showed 21 to 26 MNPCE/500 PCE. The above results are summarized in Table. 7.

TABLE 7: Summary of Results from Combination Experiments Using In Vivo Bone Marrow Micronucleu Assay

Combination of Mutagens	Remarks
DMBA + BaP (30mg/Kg) (186mg/Kg)	Antagonistic or inhibitory effect
DMBA + MMC (30mg/Kg) (1mg/Kg)	163% additive effect at 48 hr
BaP + CP (186mg/Kg) (45mg/Kg)	136% additive effect at 48 hr

PAIRWISE COMBINATIONS WITH MUTAGENS IN ABNORMAL SPERMHEAD ASSAY

Experimental mice were injected i.p. with DMBA, BaP, CP or MMC individually and also pairwise DMBA + BaP, DMBA + CP, DMBA + MMC, BaP + CP, BaP + MMC and CP + MMC. The results are summarized in the following Table 8.

TABLE 8: Pairwise Combination Experiments with Mutagens Abnormal Spermhead Assay with Mice

Mutagen	Dose mg/Kg	Mean No. Abnormal Sperms/500 Sperms ±S.E.	Remarks
DMBA	25	1.35 ± 1.08	
BaP	200	6.5 ± 2.5	
CP	45	8.7 ± 2.6	
MMC	0.8	47.8 ± 5.6	
DMBA + BaP		Toxic	Mice died
DMBA + CP		27.2 ± 4.2	Additive
DMBA + MMC		40.3 ± 6.9	Non-additive
BaP + CP		21.5 ± 7.5	Additive
BaP + MMC		Toxic	Mice died
CP + MMC		45.0 ± 4.0	Non-additive

PROMOTERS

Arochlor and Phenobarbital - Bone Marrow Micronucleus Assay

Arochlor and phenobarbital were tested against DMBA-treated mice to see whether they would act as promoters. In this case, the promutgen DMBA (25mg/Kg) was injected 24 hr before injecting the mice with either Arochlor or phenobarbital. The doses of Arochlor and phenobartibal were 0.84ml/Kg (50% of LD_{50}) and 85mg/Kg (50% of LD_{50}) respectively.

The results indicated that in Arochlor treatment the highest mean number of micronucleated PCE/500 PCE was less than 1 at 48 hr. At the same time point the sample with DMBA alone showed an average number of 5.5 micronucleated PCE/500 PCE. Arochlor plus DMBA treatment showed about 40% excess of micronucleated PCE/500 PCE over DMBA treatment alone. This result indicates that Arochlor acts as a mild promoter in the presence of DMBA. Studies with Arochlor, tested against BaP-treated mice, did not cause any promotory effect.

Phenobarbital did not show any promotory effect with DMBA. However, many mice died in the experiment when phenobarbital was used. So we had to restrict ourselves to the 48 hr samples. In general, we observed that most of the bone marrow samples had more white blood cells compared to PCE wherever either Arochlor or phenobarbital was involved.

Hexachlorobenzene - Bone Marrow Micronucleus Assay

Hexachlorobenzene (HCB, perchlorobenzene) is a chlorinated benzene of increasing toxicological and environmental concern (Morita, 52; Mumma and Lawless, 53; Quinliven et al., 54; Winteringham, 55). HCB is a hazardous component of certain industrial chemical wastes. HCB is used as a fungicide primarily to control bunt of wheat. Mismanagement of HCB wastes and use of products have resulted in several serious incidents of environmental contamination (U.S. EPA, 56).

HCB has been found in water, soil and selected aquatic organisms along the lower Mississippi River in Louisiana. Highest levels were found downstream from heavily industrialized areas (Laska et al., 57). Air immediately adjacent to production facilities has been shown to have concentrations from 1.0 to 23.6 μg/m^3.

HCB has also been identified in surface water and industrial effluents in U.S.S.R., Germany and Italy (Leoni and D'Arca, 58). HCB has been found in fish from a number of river systems in Canada (Zitko, 59). The carcinogenic activity of HCB in Syrian golden hamsters was demonstrated by Cabral et al., 60).

Mice wre injected either with DMBA (25mg/Kg) or BaP (186mg/Kg). After 24 hr these mice were injected with HCB(80mg/Kg) in DMSO and the bone marrow samples were collected at 24, 48 and 72 hr. At 24 hr, the average number of MNPCE/500 PCE was 5.8 in DMBA, 2.0 in HCB, 2.8 in BaP, and 11.0 in DMBA + HCB and 6.8 in BaP + HCB. These results indicated a promotory action of HCB against DMBA as well as BaP. However, an analysis of the results from the 48 hr samples indicated no promotory but instead an inhibitory effect. Presumably, the promoting action of HCB is evident for only 24 hr after injections and decreases after 48 hr or later (Figs. 5,6).

Hexachlorocyclohexane

Mice were treated either with DMBA (30mg/Kg) or BaP (150mg/Kg). Hexachlorocyclohexane (4mg/Kg) was injected, i.p., to the above mice after 30 minutes. Bone marrow samples were collected at 24, 48 and 72 hr. The average number of MNPCE/500 PCE were 8.4±0.9 in the 48 hr sample from the mice that received DMBA and 14.6±3.4 in the mice that received hexachlorocyclohexane. About 70% promotory effect was seen in the 48 hr sample from the mice that received both DMBA and hexachlorocyclohexane.

In the case of mice that received BaP and hexachlorocyclohexane, an 89% promotory effect was observed in the 72 hr sample, compared to the mice that received only BaP.

The above mentioned results are summarized in the following table.

TABLE 9: Potential Promoters of Mutagens
Bone Marrow Micronucleus Assay

Promoter	DMBA	BaP
Arochlor (PCB)	40% excess MNPCE	No effect
Phenobarbital	No effect	-
Hexachlorobenzene	Promotion at 24 hr Inhibiton at 48 hr	Promotion at 24 hr Inhibition at 48 hr
Hexachlorocyclohexane	70% Promotion at 48 hr	89% Promotion at 72 hr

Salmonella Typhirmurium Assay

This assay has been used to test the following potential inhibitors against BaP and MMC:- butylated hydroxyanisole, ethoxyquin, propyl gallate, ascorbic acid, caffeic acid, ellagic acid, ferulic acid, metyrapone, alpha-naphthoflavone and disulfiram. These inhibitors were tested in the presence and absence of S-9, using bacterial strain TA-98. (The procedure recommended by Ames et al. (16,17) has been followed.) However, a new Salmonella strain, TA-102, which is responsive to MMC, has been used wherever MMC is involved. Furthermore, we tested the effect of BaP, DMBA and CP using TA-102 strain in the presence and absence of S-9.

TA-102 is a new strain which was constructed primarily for detecting mutagens that require an intact excision repair system. TA-102 contains the R-factor plasmid, pKM101. It also contains the multicopy plasmid, pAQ1, which carries the G428 mutation and tetracycline resistance gene. TA-102 contains the ochre mutation $\frac{-TAA-}{-ATT-}$ in the his G gene. This strain detects efficiently a variety of mutagens such as formaldehyde, glyoxal, various hydroperoxides, bleomycin, phenylhydrazine, X-rays, UV light, streptonigrin and cross-linking agents, such as psorolens and mitomycin C (Maron and Ames, 61). (All the bacterial test strains have been kindly supplied by Dr. B.N. Ames.)

Effect of Butylated Hydroxyanisole (BHA) Against BaP

5μg of BaP have been used per plate in the controls as well as against BHA. Average number of revertants per plate were 193±7. BHA at the highest dose of 250μg showed toxicity. BHA showed an inhibitory effect at the highest non-toxic dose of 125μg/plate. Since the 250μg dose showed toxicity, 125μg may be, in fact, toxic and was not detected. Therefore, the amount of BHA was reduced very much in further experiments and the concentrations ranged between 15μg - 120μg/plate. Average number of revertants were 109±8 with 15μg, 65±3 with 30μg, 123±12 with 60μg and 112±5 with 120μg/plate of BHA. In this experiment, BaP controls gave an average number of revertants of 88±9. If we assume that the inhibitory effect has been seen with 30μg dose of BHA, the inhibitory effect should be almost doubled at 60μg dose. This was not the case. Hence it has been decided that BHA is not an inhibitor against BaP in the Salmonella assay.

Effect of Ethoxyquin Against BaP

BaP controls in this experiment gave 134±2 revertants on average. 125µg, 250µg, 500µg and 1000µg of ethoxyquin doses were tested against BaP using TA-98 strain. The average number of revertants per plate were 134±17 with 125µg, 106±3 with 250µg, and 90±11 with 500µg. No revertants were noted with 1000µg. The last two doses were found to be toxic. On the whole, no inhibitory effect of ethoxyquin was noticed.

Effect of Propyl Gallate Against BaP

In this experiment, BaP controls gave 145±11 revertants on average. 125µg, 250µg, 500µg and 1000µg doses of propyl gallate were tested against BaP. Highest dose of 1000µg/plate was found to be toxic. The average number of revertants per plate were 127±12 with 125µg, 148±9 with 250µg and 121±9 with 500µg/plate. These results indicate that propyl gallate is not an inhibitor against BaP in this assay.

Effect of Ascorbic Acid Against BaP

BaP controls gave 133±7 revertants on the average. The doses of ascorbic acid were 15, 30, 60 and 120µg/plate. The average number of revertants were 84±5 with 15µg, 89±1 with 30µg, 126±8 with 60µg and 117±3 with 120µg/plate. Ascorbic acid, by itself, gave 50±10 revertants with 120µg dose. On the whole, some inhibitory effect has been noticed.

Results with caffeic acid, ellagic acid, metyrapone, ferulic acid, alpha-naphthoflavone and disulfiram showed varying inhibitory effects in the Ames Salmonella assay against TA-98 bacterial strain. The results are presented in Table 10.

TABLE 10: Effect of Chemical Inhibitors Against BaP Salmonella Typhimurium Assay with TA-98 and S-9

Chemical	Maximum Dose	Revertants/Plate $\bar{X}$ ± S.E.	% Inhibition
BaP	5 μg	135±4	
Caffeic acid	4000nmol	83±5	40
Ellagic acid	4000nmol	80±13	41
Metyrapone	40μg	34±7	75
Controls			
DMSO	0.1ml	43±3	
Caffeic acid	4000nmol	27±2	
Ellagic acid	4000nmol	18±3	
Metyrapone	40 μg	31±3	
BaP	5 μg	185±9	
Ferulic acid	4000nmol	127±6	31
α-Naphthoflavone	40 μg	93±9	50
Disulfiram	40 μg	85±8	54
Controls			
Ferulic acid	4000nmol	53±2	
α-Naphthoflavone	40 μg	52±6	
Disulfiram	40 μg	53±3	
DMSO	0.2ml	61±6	

The following conclusions could be drawn from the above results.

1) Average number of revertants/plate with BaP alone varied from 135 to 185 in two different experimental runs. Reversion rate to TA-98 is about 40, so BaP has shown mutagenicity, with an increase in the reverion rate of 3 to 4 times.

2) An inhibitory effect was shown by all potential inhibitors.

3) The dose-response of the chemicals was not linear.

The above results with the Salmonella assay and the *in vivo* bone marrow micronucleus and abnormal sperm assays have provided clues to the possible inhibitory action of chemicals that are antioxidants, phenols and sulfur-containing compounds.

BaP, CP, DMBA and MMC were tested with and without the presence of S-9 using TA-102 bacterial strain. BaP gave negative resonse without S-9 and positive repsonse with S-9. CP and DMBA showed negative response with and without S-9. MMC showed a linear response on TA-102 with and without S-9. However, the response without S-9 was higher than that with S-9.

Caffeic acid, metyrapone, α-naphthoflavone, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ellagic acid, ascorbic acid, propyl gallate, ferulic acid and ethoxyquin were tsted against MMC using TA-102. None of the above mentioned putative inhibitors showed any inhibitory action against MMC.

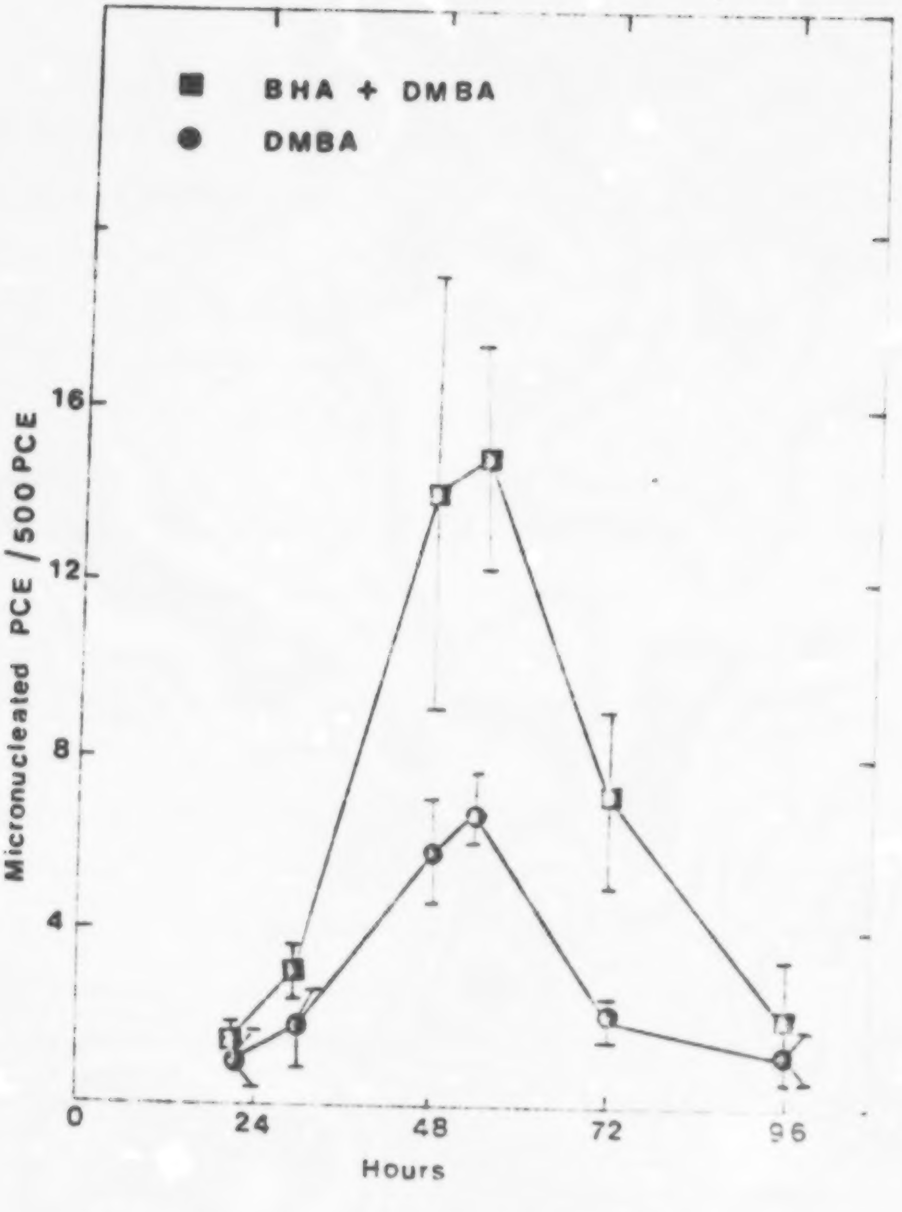

Fig 1. Results of bone marrow micronucleus assay of mice fed a powdered food mixed with butylated hydroxyanisole (BHA) and treated i.p. with DMBA in DMSO. BHA in the presence of DMBA acted as a promoter.

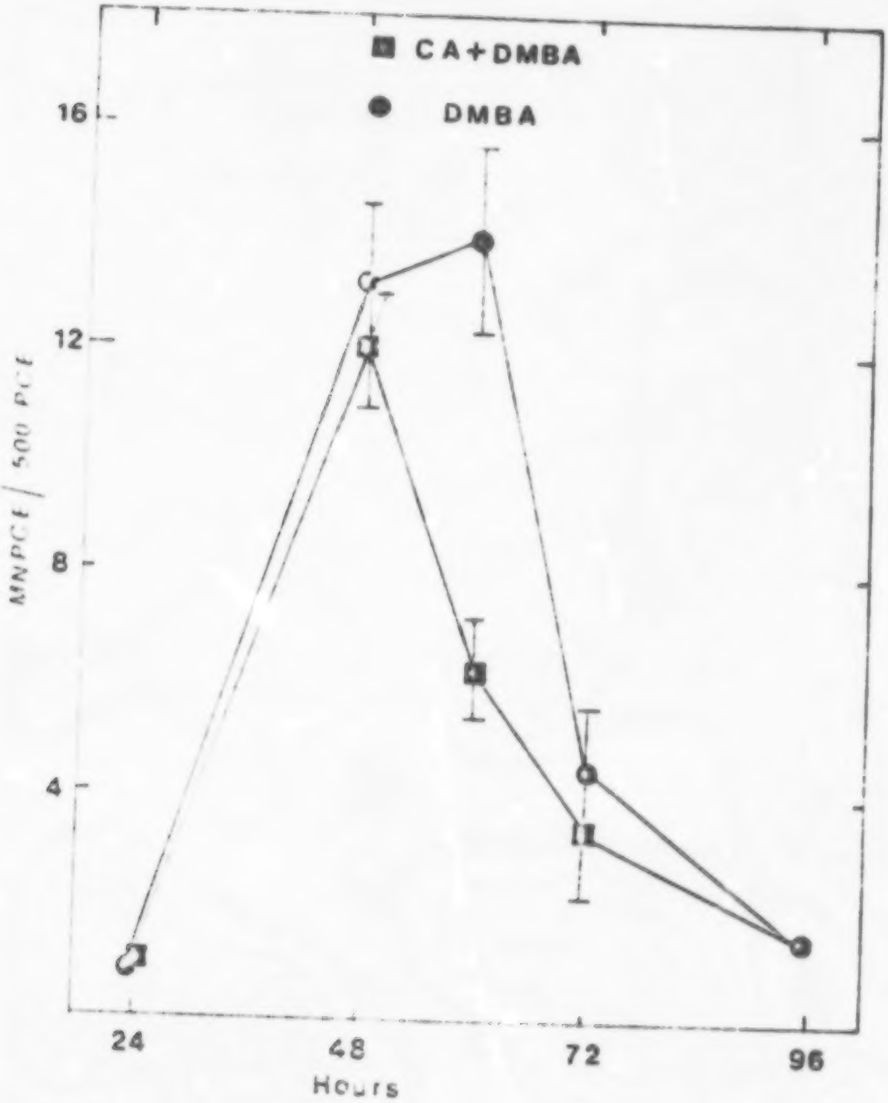

Fig. 2. Results of bone marrow micronucleus assay of mice fed a powdered food mixed with 2% caffeic acid and treated i.p. with DMBA in DMSO. Caffeic acid (CA) inhibits action of DMBA by about 50% at 60 hr sampling time.

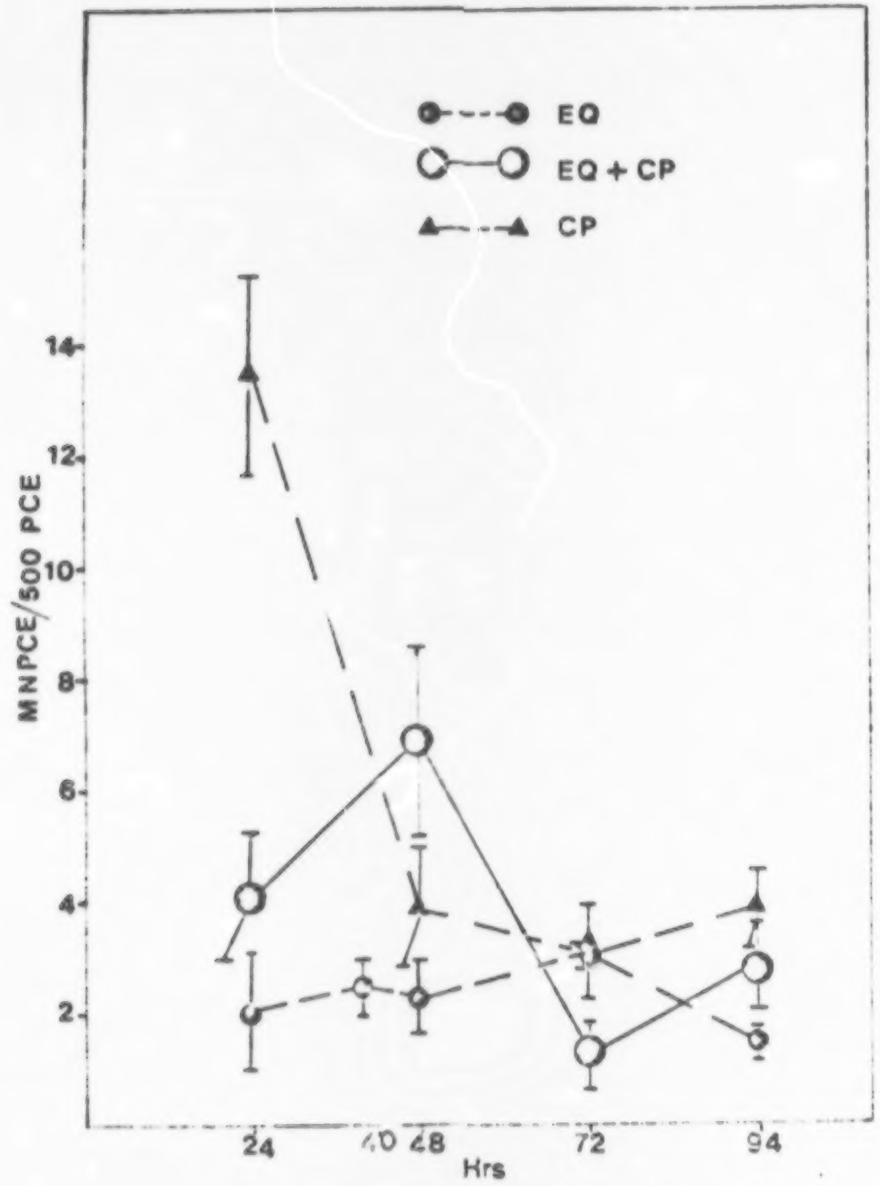

Fig. 3. Results of bone marrow micronucleus assay of mice fed a powdered food mixed with ethoxyquin (EQ) and treated with cyclophosphamide (CP).

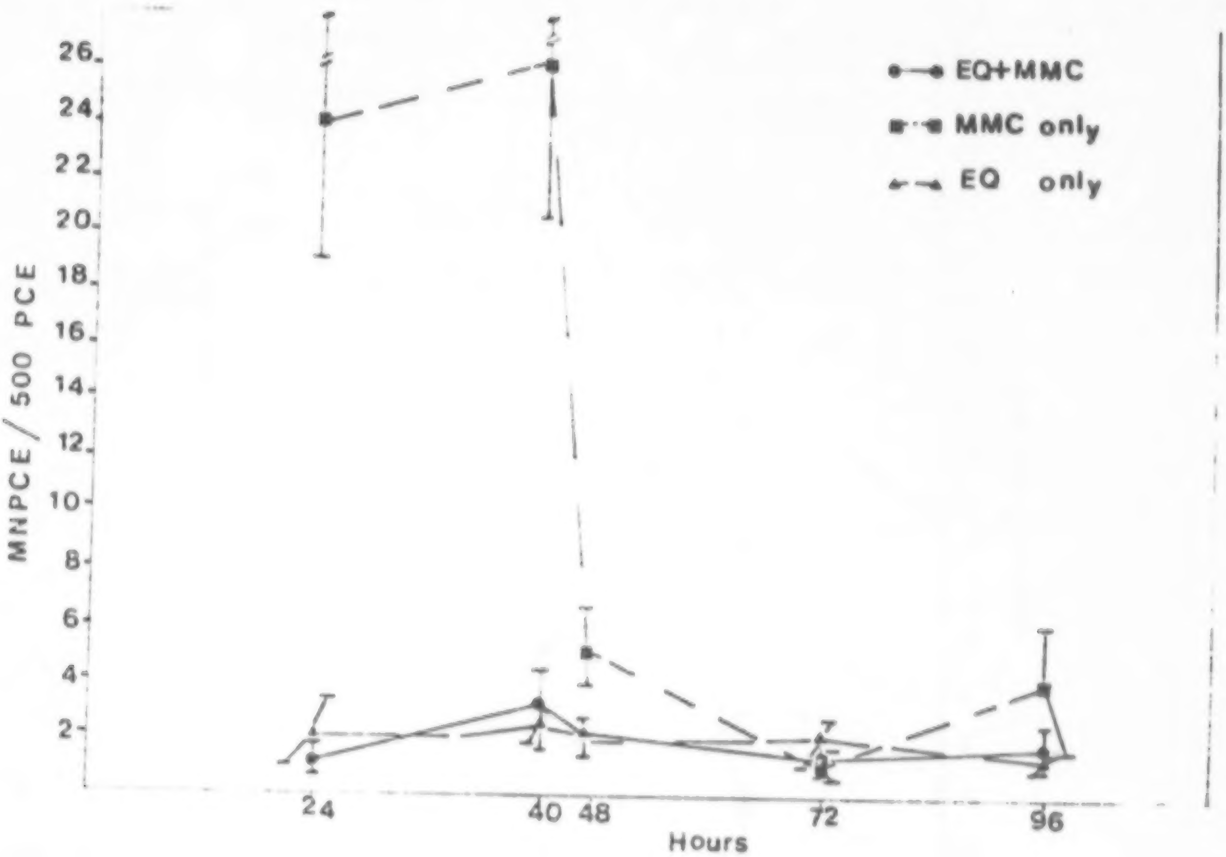

Fig. 4. Results of bone marrow micronucleus assay of mice fed a powdered food mixed with ethoxyquin (EQ) and treated with mitomycin C (MMC).

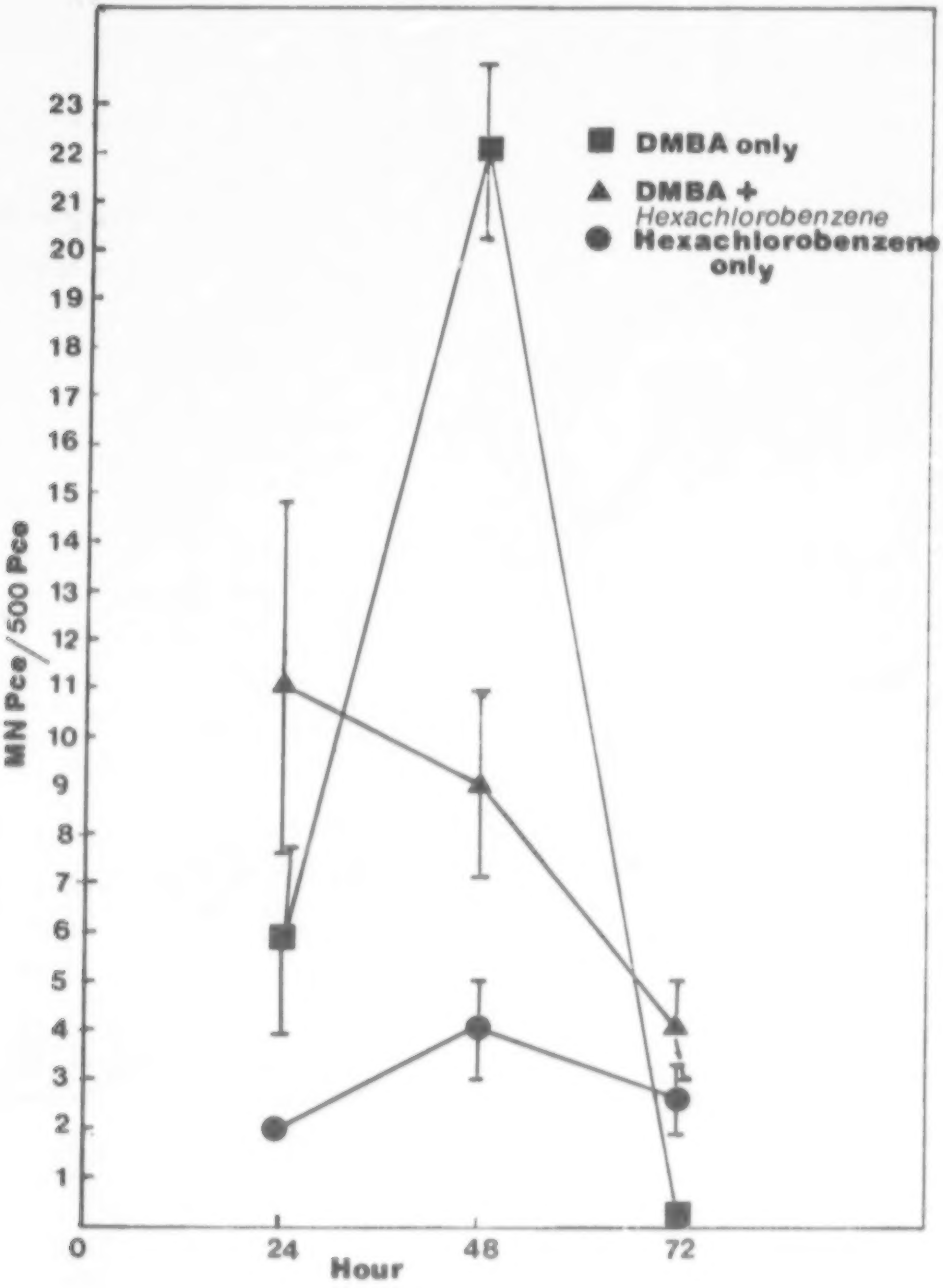

Fig. 5. Results from bone marrow micronucleus assay in mice treated with DMBA and hexachlorobenzene.

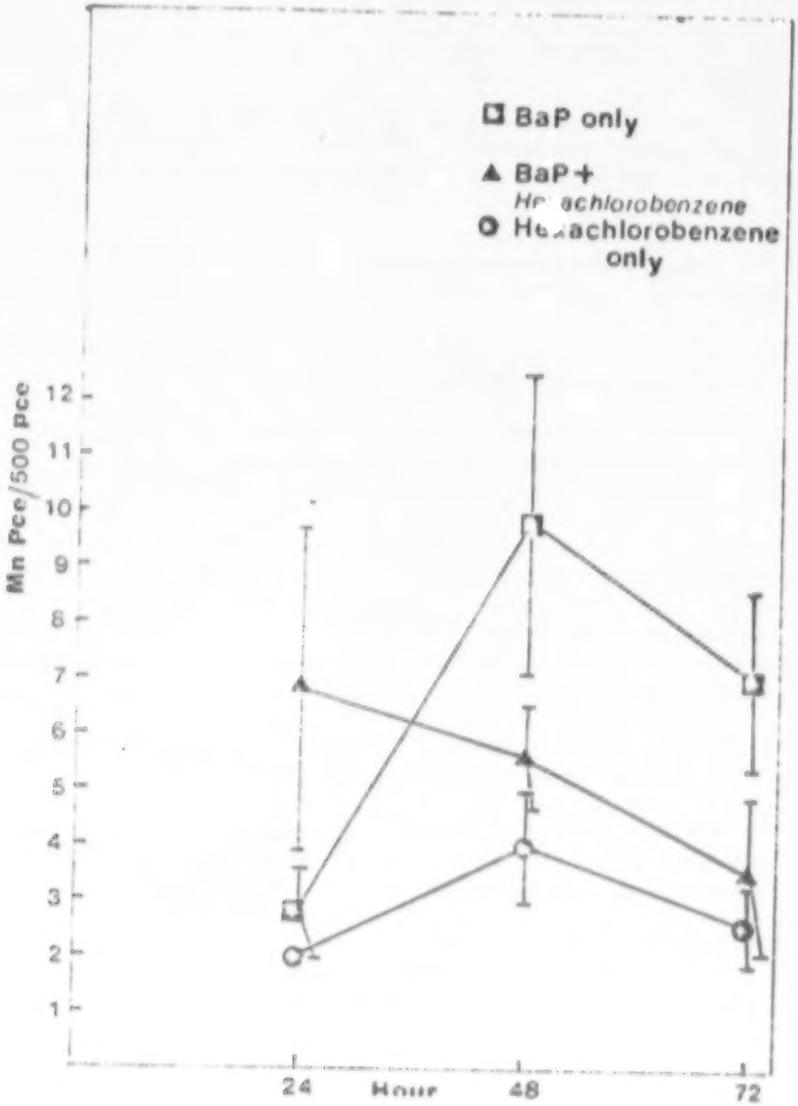

Fig. 6. Results from bone marrow micronucleus assay in mice treated with BaP and hexachlorobenzene.

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Collaborative Study on Short-Term Tests for Genotoxicity and Carcinogenicity

D.M. Logan [1]
and
M.F. Salamone [2]

[1] Department of Biology, York University, **North York, Ontario, M3J 1P3**

[2] Ministry of the Environment, **Biohazards Unit, P.O. 213, Resources**
Road, Rexdale, Ontario, M9W 5L1

Introduction

In 1976 the Health and Safety Executive (HSE) and the Medical Research Council (MRC) of the United Kingdom commissioned a programme to develop short-term mutagenicity tests to screen for possible carcinogens. Shortly thereafter, scientists from Imperical Chemical Industries (ICI) joined the programme and these groups agreed upon a combined approach. They believed that the best method of comparing the utility of different assays would be to test pairs of structurally related compounds, one carcinogenic, the other not, in the assay systems being assessed.

ICI agreed to supply the purified compounds and they selected 25 carcinogens and 17 non-carinogens, which included 14 paired compounds, for the study.

Since the available quantity of each chemical exceeded the requirements of the programme, the National Institute of Environmental Health Science (NIEHS) in the U.S.A. was invited to join the study with the intention of providing a wider variety of mutagenicity test systems. With the subsequent inclusion of several Japanese laboratories under the auspices of Japan's National Cancer Center Research Institute, the first international study on mutagenesis/carcinogenesis was initiated.

In the ensuring years, 42 test compounds were tested on 35 assay systems in some 60 laboratories. The study produced several results, including the identification of several previously unsuspected carcinogens, and tested the efficacy of different batteries of short-term mutagenicity tests. Unfortunately, the range of results with certain chemicals and assays systems precluded the identification, at that time, of a single battery of short-term tests which would provide unequivocal identification of carcinogens. The other successes of the study, however, led to the establishment of a second international study.

This latter study, in which we are participants, is designed to correct the shortcomings of the initial study and expand its scope. The principal scientists from ICI and NIEHS who were originally involved in the organization and direction of the initial study are associated in a similar capacity with the present programme. This study, however, involved many additional nations and laboratories and the study is now jointly sponsored by the World Health Organization (WHO), the United Nations Environment programme and the International Labour Office.

Our participation involves testing four assay systems: the *in vivo* mammalian bone marrow micronucleus assay, the *in vivo* mammalian abnormal spermhead assay, the *in vitro* sister chromatid exchange assay and the replicative/killing assay. Four to six chemicals were to be tested on the two *in vivo* assays and ten chemicals on the *in vitro* assays. In addition, six chemicals, which were not part of the international study, were supplied by the Ontario Ministry of the Environment for testing on the *in vivo* assays.

Materials and Methods

Chemicals

(a) WHO Chemicals

All those chemicals which formed part of the international study were synthesized and provided in pure form by ICI. In each case, all testing labs received samples from a single synthesis to ensure comparability.

(b) MOE Chemicals

All chemicals provided by the MOE were obtained from commercial suppliers in the purest form commercially available.

All chemicals were dissolved or diluted in dimethyl sulfoxide. All other chemicals were obtained from regular suppliers in the highest purity available.

Animals

The B6C3F1 hybrid mouse was used in both *in vivo* assays. All mice were purchased from Canadian Breeding Farm and Laboratories Limited, Quebec. Mice used in a particular experiment were always selected from a single shipment and were age matched in all cases.

Chinese Hamster Ovary (CHO) cells were kindly supplied by Dr. Richard Marshall of York University.

Assays

In vivo

Bone Marrow Micronucleus Assay

Micronuclei are small pieces of nuclear material found in the cytoplasm of dividing cells. The material arises primarily from chromosome fragments that are not incorporated into daughter nuclei at the time of cellular division. Clastogens are mutagenic agents which are capable of breaking chromosomes and producing micronuclei, and thus are detectable by a micronucleus assay. The micronucleus assay used in this study was a slight modification of the mammalian bone marrow micronucleus assay described by Salamone *et al* (1980) and Salamone and Heddle (1983) in which polychromatic erythrocytes are used as the target cells. Mice were treated once or on two consecutive days with a given dose of the test chemical. Approximately 24, 48 and 72 hr after the final treatment, samples were taken to determine the frequency of micronucleated bone marrow polychromatic erythrocytes.

For the WHO chemicals, a series of doses was tested with each chemical (rather than a single dose) to provide dose-effect data.

An equal number of male and female mice of the same age were used for each treatment. Samples were taken by first sacrificing the mice by cervical dislocation, removing a femur and stripping it of muscle (Salamone et al, 1980). The bone marrow was then removed by making a small opening at the iliac end of the femur and introducing the pointed shaft of a 2.5 cm pin into the femur at the epiphysial end. As the pin was slowly pushed and twisted into the marrow canal, the marrow exuded out the hole at the iliac end. The marrow was placed on a clean microscope slide and a drop of fetal bovine serum was then added. Using the edge of a clean slide, the marrow was mixed with the serum until homogenous and then spread as a smear. These bone marrow slides were then fixed in absolute methanol and stained with a 5% solution of Giemsa (BDH) (Salamone et al, 1980). The number of micronucleated polychromatic erythrocytes (PCE) per 500 PCE was then determined for each slide and results from mice treated with the test chemical were compared with those from non-treated and solvent treated mice.

Spermhead Abnormality Assay

The assay used in this study was that recommended by Wyrobek et al (1983). Male mice 11 weeks or older were given daily interperitoneal injection of the test chemical for five days. The protocol included five or six treatment groups plus zero, and solvent control groups. The effectiveness of the assay was routinely monitored by the injection of parallel experiments of known mutagens. Thirty-five days after the final treatment, the mice were sacrificed by cervical dislocation and both epididymides removed and placed in phosphate buffer. The tissue was minced and then stained for 20 min in a 1% Eosin solution. Stained sperm were spread on microscope slides, allowed to air dry and mounted with DPX (BDH Chemical). The number of abnormal sperm per 500 sperm was then measured.

In Vitro

Sister Chromatid Exchange (SCE)

The SCE assay monitors the frequency of exchange between chromatid arms of the same chromosome. Each sister chromatid can be selectively stained so that one appears dark while the other appears light. Exchanges can then be easily recognized. For this study, Chinese Hamster Ovary (CHO) cells were used and the basic method followed was that described by Latt et al (1981). CHO cells were routinely cultured in Eagles MEM buffered medium (DIFCO) supplemented with 10% fetal calf serum.

For testing purposes, culture flasks were initially seeded with 1.5×10^5 cells and allowed to grow to approximately 75% confluence. The cells were then freed and plated on 60 mm plates at 4.8×10^5 cells per plate and allowed to grow overnight. The old medium was then removed and fresh serum-free medium containing the test chemical or the test chemical plus 59 (metabolic activation mixture) was added. The cultures were incubated 1 h at 37°C following which the medium was removed, the cells washed with Hank's BSS (Gibco) and then recovered with fresh medium containing 20 μM BUDR (bromodeoxyuridine).

Twenty-four hours after the addition of BUDR, 0.1 ml of colchicine (10^{-4} M) was added. Two hours later, the BUDR colchicine medium was removed and the cells were harvested. Cells were trypsinized and 74 mM KCL was added for 6 min. The cells were then washed 3 times in Carnoys' (glacial acetic-alcohol) fixative, stained with Hoechst for 12 min, illuminated 22 h and then stained 5 min with 20% buffered Giemsa. The average frequency of SCE per 25 complete sets of metaphase chromosomes were scored. Results from cells treated with the test chemical were compared with those from non-treated or solvent treated cells.

Replicative/Killing Mutagenicity Assay

The Replicative/Killing assay measured the mutagenicity of chemical compounds using a genetically engineered strain of the bacterium *Escherichia coli*. This particular bacterial strain contains an incomplete virus particle which, in the inactive state at 30°C, resides symbiotically in the bacterial cell. Activation of the virus can be achieved by an increase in the temperature of the culture to 42°C and this activation ultimately kills the bacterial cell. Mutagenic agents, on the other hand, may cause damage to the viral genetic material and prevent either its activation or expression. Bacterial cells of this strain, in which the virus has been mutated, will not be killed by a temperature shift and will develop into colonies.

In the Replicative Killing assay, a culture of bacterial cells is mixed with the test chemical, both in the presence and absence of metabolic activation enzymes (S9). A range of concentrations of the chemical is tested. Following a suitable incubation period, aliquots of the bacterial suspension are plated on a nutrient agar medium which is then incubated for 48 hr at 42°C. Bacterial colonies developing on the plates are counted and compared to counts on control plates containing a similar bacterial suspension which was not exposed to the test substances. A chemical possessing mutagenic properties should induce a dose-related increase in surviving colonies which exceeds, by a minimum of two fold, those on control plates.

LD_{50} and Toxicity

To minimize the frequency of false negative results, it is desirable to test chemicals at the highest tolerable dose. This dose is generally determined from the toxicity of the chemical, toxicity being expressed as some lethal dose percentage (e.g., LD_{10}, LD_{30}, LD_{50}), i.e., that dose which is expected to cause the death of a given percentage of the population of test organisms. In this report, the toxicity in the in vivo assays is expressed as the LD_{50}, while for the in vitro studies, a visual measure of cell growth inhibition was used to ascertain the maximum test dose, i.e., the lowest dose at which cell growth inhibition was clearly discernible.

LD_{50}'s were calculated from the tables and formulae of Weil (1952). Each compound was tested at four or five doses, with the doses being separated by a constant factor (usually between 1.2 and 2). A uniform number of mice (usually 3 to 6) were injected at each of the selected doses and the number of deaths at each dose was determined daily over a 7 day period. For the micronuclei assay, the maximum dose tested was generally 80% of the LD_{50}, while for the abnormal spermhead assay 50% of the LD_{50} was routinely the maximum dose.

For the sister chromatid exchange assay, the toxicity of each chemical was checked using micro- and millimolar concentrations. A uniform number of CHO cells was allowed to grow in each well of a deep well plate (12 wells/plate). Various concentrations of the test substance were then added to each well. After 24 h each well was visually checked to determine the relative density of the cell growth. The lowest dose which exhibited some degree of toxicity was selected as the maximum test dose.

Toxicity with the RK assay is inherent within the assay as explained above.

Results and Discussion

The approximate LD_{50}'s of the chemicals listed in the *in vivo* assays are presented in Table 1. In interpreting this data, however, several points must be considered--most importantly, solvent effects. Many organic solvents are toxic in animal systems and thus, unless a chemical can be easily dissolved in water or physiological saline, dimethylsulfoxide (DMSO) becomes the solvent of choice. DMSO, which is a good universal solvent, is only mildly toxic to animals; however, it is not necessarily the best solvent for certain substances. For example, in the cases of atrazine and mirex, it was difficult to administer enough chemical to the animal in diluted form as DMSO was not a good solvent. In these cases, slurries of the test chemical and DMSO were injected. This required larger bore needles than usual and may have resulted in significant loss of chemical either from loss at the wound site or from retention in the syringe. Thus, for these two chemicals in particular, only estimated limits for the LD_{50} are reported.

It is of interest to note that the analogs, 2-acetylaminofluorene (2AAF) and 4-acetylaminofluorene (4AAF), have strikingly different LD_{50}. 2AAF has been reported to be the mutagenically more active of the two compounds, both *in vitro* and *in vivo*, and yet it was many times less toxic.

A summary of the *in vivo* mutagenic results for both the bone marrow micronucleus and the abnormal spermhead assays is presented in Table 2. In those cases where the data are equivocal, confirmation assays are underway.

TABLE 1: LD_{50} TEST RESULTS ON CHEMICALS TESTED IN VIVO

Compound	Approximate LD_{50}
WHO Compounds	
4-Acetylaminoflourene	364 mg/Kg
2-Acetylaminofluorene	2,220 mg/Kg
Benzo(a)pyrene	232 mg/Kg
Pyrene	514 mg/Kg
Hexamethylphosphoramide	
O-Toluidine	
MOE Compounds	
Atrazine	300-700 mg/Kg [a]
Chlorobenzene	65 mg/Kg
Ethylene dichloride	∿250 mg/Kg
Mirex	150-400 mg/Kg [a]
Pentachlorophenol	46 mg/Kg
Trichloroethylene plus stabilizer	125 mg/Kg
Trichloroethylene minus stabilizer	60-100 mg/Kg [b]

[a] Due to solvent difficulties, it has not yet been possible to determine a narrower toxicity range.

[b] Toxicity testing incomplete.

TABLE 2: SUMMARY OF IN VIVO MUTAGENICITY TEST RESULTS

Compound	Assay	
	Micronucleus	Abnormal Spermhead
WHO Compounds:		
4-Acetylaminofluorene	TBS	-
2-Acetylaminofluorene	TBS	+ [a]
Benzo(a)pyrene	+	TBS
Pyrene	-	?+
Hexamethylphosphoramide	+	NT
O-Toluidine	?+	NT
MOE Compounds:		
Atrazine	INC	INC
Chlorobenzene	- [a]	-
Ethylene Dichloride	+ [a]	-
Mirex	- [a]	TBS
Pentachlorophenol	+ [a]	-
Trichloroethylene plus stabilizer	-	-
Trichloroethylene minus stabilizer	INC	INC

[a] Unconfirmed

NT No test, test not conducted as these chemicals were dropped from WHO programme.

TBS To be scored, experiment finished but slides not scored.

INC Incomplete, a full test has notbeen conducted

+ Positive

- Negative

?+ A weak positive response, requires confirmation at higher doses.

For the WHO compounds, micronucleus assay results are available for four of the compounds and three results are available based on the sperm assay (one compound was tested in both). Benzo(a)pyrene (BaP), hexamethylphosphoramide (HMPA) and O-toluidine gave positive results in the micronucleus assay while pyrene gave a negative result, i.e., pyrene is non-clastogenic. The data obtained with both latter compounds, i.e., O-toluidine and pyrene, are, however, not completely conclusive and require confirmation.

In the other in vivo assay system, i.e., abnormal spermhead assay, 2AAF gave positive results (i.e., acted mutagenically) while 4AAF did not. Pyrene, at high doses, also gave positive results (note: contrast with micronucleus assay) but again these results are equivocal and subject to confirmation.

In in vitro assay systems, both benzo(a)pyrene and pyrene have been reported to give positive responses, i.e., mutagenic with BaP producing the stronger response (de Serres and Ashby, 1981). In vivo assays have identified BaP as a fairly strong mutagen but pyrene has not been detected as a mutagen until now. The results presented here suggest that pyrene may indeed induce in vivo mutagenesis in gonadal tissue. These results need verification, but it is of interest to note that Topham (1981) reported an almost identical response with pyrene on the sperm assay.

2AAF and 4AAF have produced mutagenic patterns very similar to that of BaP and pyrene, respectively. The abnormal spermhead results for these compounds are, therefore, not totally unexpected.

HMPA has not produced a positive mutagenic response in *in vitro* systems but has produced strong mutagenic results *in vivo* (de Serres and Ashby, 1981). The micronucleus data presented here verify HMPA's mutagenic response *in vivo*. In contrast, O-toluidine has, in the past, been reported to produce positive results in *in vitro* assays, but negative results in *in vivo* assays (i.e., a response pattern equivalent to that shown by pyrene and 4AAF). In our hands, however, O-toluidine produces a weak positive response in an *in vivo* assay. Confirmation of this result will reflect well on the current protocol for the micronucleus assay. Unfortunately, during the course of our work, both O-toluidine and HMPA were dropped from the WHO programme and work on these compounds will accordingly be delayed.

Of the seven MOE compounds tested, only 1,2-dichloroethane and pentachlorophenol have so far shown positive clastogenic responses, and none of the compounds appear to be mutagenic to gonadal tissue. Most of the micronucleus assay data the subject to confirmation, however. Furthermore, initial tests on atrazine and trichloroethylene minus stabilizer have not been completed, therefore, it is possible that one or two other of these chemicals may prove mutagenic in *in vivo* systems.

The *in vitro* results are presented in Table 3. Since the metabolic enzymes necessary for the conversion of promutagens into mutagens are not inherently present in CHO cells or *E. coli*, they must be added to the reaction mixture. Each chemical was tested with (+S9) or without (-S9), the activation enzymes.

TABLE 3: SUMMARY OF IN VITRO MUTAGENICITY TEST RESULTS

Compound	Assay			
	Replicative/killing		Sister Chromatid Exchange	
	-S9	+S9	-S9	+S9
2-Acetylaminofluorene	-	+	-	+
Benzoin	-	?$^+$	TBS	TBS
Caprolactam	-	-	TBS	TBS
Hexamethylphosphoramide	INC	INC	-	TBS
Safrole	?$^+$	?$^+$	TBS	TBS
O-Toluidine	-	-	-	TBS
2-Aminoanthracene	-	+	-	+
N-methyl-N-nitronitrosoguanidine	+	NT	+	NT

TBS	To be scored.
INC	Incomplete, a full test has not been conducted.
+	Positive
-	Negative
?$^+$	A weak positive response, requires confirmation at higher doses.
NT	Not tested.
±S9	With or without metabolic activation enzymes.

Two of the chemicals for which results are given in Table 3 were not part of the International Program, but were added on our part to provide controls on the efficacy of the assays and the S9 response. N-methyl-N'-nitronitrosoguanidine (MNNG) is a direct-acting compound which should induce mutagenic changes in the absence of S9. This was confirmed in both assays. 2-aminoanthracene (2AA), on the other hand, requires S9 for the production of its mutagenically active intermediate. With both assays, 2AA gave a negative mutagenic response in the presence of S9. Thus, the S9 enzymes and the assays were responding normally.

Of the six other chemicals listed in Table 3, 2AAF, HMPA, safrole and O-toluidine would be expected to produce positive mutagenic data, primarily in the presence of S9, while benzoin and caprolactum should produce non-mutagenic responses. However, since the results are complete only for 2AAF, it is difficult to interpret accurately the response observed.

2AAF was positive in both assays in the presence of S9 and negative in both assays in the absence of S9, as was expected. Safrole produced a weak unconfirmed positive result on the RK assay. Since Safrole is known to be a potent carcinogen, a positive mutagenic result would be expected. On the other hand, O-toluidine was negative on the RK assay in the presence or absence of S9 and benzoin produced unconfirmed weak positive results in the presence of S9. Those results (if confirmed) may indicate that the RK assay does not discriminate well between positively and negatively mutagenic compounds. However, no current assay is completely effective in such discrimination, and further comparative studies will be required to assess the effectiveness of these assay.

Because of the sparcity of data with the SCE assay, for which testing has only recently begun, it is difficult to identify any particular response pattern at this time.

This paper should be viewed as a progress report and only when final data are available from all the participating laboratories will more extensive conclusions be warranted in regard to both the chemicals tested and the utility of various assay systems.

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SUPPRESSION OF IMMUNE DEFENCES BY HALOGENATED AROMATIC HYDROCARBONS

by David A. Clark, George Sweeney, and Jack Gauldie, McMaster University
Departments of Medicine and Pathology, Hamilton, Ontario.

Introduction:

Industrialized communities, particularly in the region of the great lakes, recognize the potential threat to health posed by halogenated aromatic hydrocarbons in the environment. Polychlorinated biphenyls (PCBs) are endemic in southwestern Ontario and certain of the great lakes are significantly contaminated. A large reservoir of TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) on Grand Island, New York, threatens Lake Ontario, and electric transformers and waste dumps elsewhere are of concern. The US Government resettled an entire community in a town where the roads had been sprayed with oil inadvertently contaminated with dioxin (TCDD) (1). It is estimated that 70-80 % of the population of Michigan harbours measurable levels of polybrominated biphenyls (PBBs) as a result of accidental contamination of cattle feed (2). Once any of these fat-soluble, poorly metabolized compounds enters the food chain, a wide variety of species may be subject to chronic exposure, including humans.

TCDD or dioxin (D1746016G), one of the most toxic of the halogenated aromatic hydrocarbons, has been the most extensively studied and provides a prototype model for haloaromatic toxicity. As illustrated in Table 1, toxic effects may be observed in a variety of organs (3,4). Susceptibility to toxicity requires the presence of a receptor protein to which TCDD binds in the cell (5-7). The level of the receptor is genetically controlled and binding of haloaromatic compounds such as PCBs and PBBs to the receptor is also associated with their ability to produce lesions similar to those that occur with dioxin (TCDD) (5-7). The major toxic effects recognized to date in humans exposed to TCDD (*) include skin lesions, liver damage and porphyria, neuropathy and neurasthenia, and hyperlipidemia (3,8). There is suggestive evidence for an increase in soft tissue sarcomas (9,10) and an increased tumor incidence has been seen in exposed animals (3). Considerable variation exists among different species of animals in susceptibility and pattern of tissue damage that develops following TCDD (3,4,11,12). Many species, for example, do not develop skin lesions. A common feature seen in all animal species which have been studied is atrophy of the thymus gland. Indeed TCDD-treated guinea pigs that die rapidly after very low doses of TCDD show thymus atrophy as the main lesion.

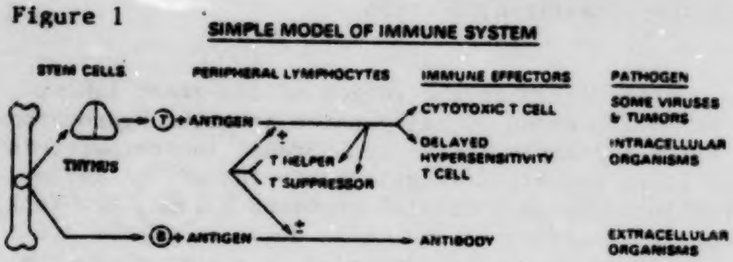
TABLE I

SPECTRUM OF LESIONS PRODUCED BY TCDD (HUMAN* AND ANIMAL)

Chloracne*, Pigmentation*, Hirsutism*
Liver damage* and phorphyria*
Neuropathy* and neurasthenia*
Hyperlipemia*
Embryotoxicity and malformations
Carcinomas and soft tissue sarcomas*?
Atrophy of thymus and increased sensitivity to bacterial toxins
Edema and pericardial effusion
Death

The thymus gland is a key organ in the immune defence system and produces at least four different types of blood lymphocytes (peripheral thymus-derived cells or T cells) that mediate resistance to infectious agents in the environment. As illustrated in figure 1, stem cells from bone marrow emigrate to thymus where they develop into cells that react to the alien (i.e. antigenic) determinants on foreign bacteria. On exposure to antigen, T cells undergo further development into different types of effector cells. Cytotoxic T cells (CTL) lyse virus infected cells and certain types of tumor cells associated with herpes-type viruses such as the highly aggressive and fatal Kaposi's sarcoma seen in immunodeficient men and women (13,14). T cells which produce delayed hypersensitivity reactions (classical cell-mediated immunity) produce soluble mediators that activate macrophages. This activation is crucial if the macrophage is to deal effectively with certain types of intracellar agents (15) such as the tuberculosis and leprosy organisms. Activated macrophages may also play a role in resistance to some types of tumors (16). Two other types of T cells produced by the immune response serve to regulate the response by either increasing ("help") or inhibiting ("suppression") the production of effector cells (17). Stem cells from the bone marrow also develop independently of the thymus into antibody producing cells. Antibody is important in resistance to extracellar parasites. Since the antibody response is regulated by helper and suppressor T cells, however, a toxic effect of TCDD affecting production of helper and suppressor cells by the thymus could potentially affect all mechanisms of immune defence.

Figure 1


SIMPLE MODEL OF IMMUNE SYSTEM
STEM CELLS
PERIPHERAL LYMPHOCYTES
IMMUNE EFFECTORS
PATHOGEN
THYMUS
T + ANTIGEN
B + ANTIGEN
T HELPER
T SUPPRESSOR
±
±
CYTOTOXIC T CELL
DELAYED HYPERSENSITIVITY T CELL
ANTIBODY
SOME VIRUSES & TUMORS
INTRACELLULAR ORGANISMS
EXTRACELLULAR ORGANISMS

Over the past four years, we have conducted an extensive series of investigations in order to better understand what effect TCDD and related haloaromatic hydrocarbons might have on immunity and disease susceptibility. We have employed laboratory mice for these studies as their immune system is similar to that of man and genetically pure strains were available that differed in their susceptibility to TCDD (6,7). The objective of this paper is to summarize the results from these studies, to define problems requiring further investigation, and to outline insights gained concerning the problem of environmental exposure of humans to TCDD.

Type of immune dysfunction produced by TCDD in mice:

The design of the experiments is illustrated in figure 2. Following a series of 4 weekly doses of TCDD by injection, the animals were either challenged with antigen in vivo (as illustrated), or the cells removed from lymph nodes, spleen, and thymus for study in vitro. The in vivo assay system showed that the generation of cytotoxic T cells (CTL) was particularly sensitive to suppression by TCDD (Table II) and the mechanism of this effect was further analysed in vitro as shown in figure 3. Cell suspensions prepared from the lymph nodes, or spleen of normal and TCDD-treated mice, were cultured in test tubes with stimulator cells bearing the H-2^d alloantigen and the number

Figure 2

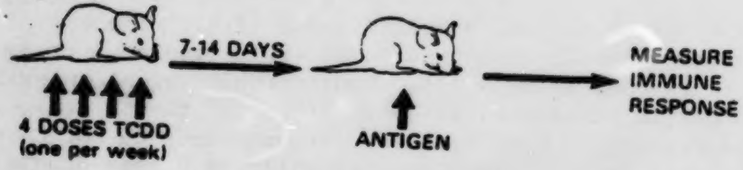
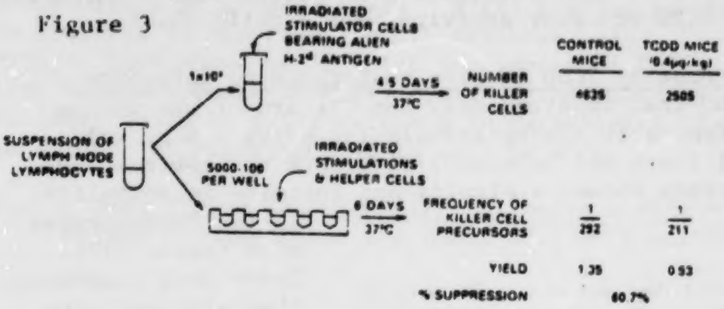

7-14 DAYS
4 DOSES TCDD
(one per week)
ANTIGEN
MEASURE IMMUNE RESPONSE

Table II

	DOSE OF TCDD (μg/kg over 4 weeks)				
	0.4	4	40	100	400
1. Appearance	Healthy	Healthy	Healthy	Sick	Sick
2. Cells in Lymphoid Organs					
Spleen*& Lymph Nodes				↓	↓↓
Thymus		↓	↓↓	↓↓↓	↓↓↓
3. Type of Immune Response					
Antibody			↓↓ ±	ND	ND
Delayed Hypersensitivity		↓	↓	ND	ND
Cytotoxic T Cell	↓↓	↓↓	↓↓	ND	ND

of CTL produced against H-2^d was determined by measuring lysis of antigen-bearing target cells (% ^{51}Cr-release cytotoxicity assay). A typical result illustrated to the right in the upper panel of the figure shows that 10^6 lymph node cells from TCDD-treated mice produced only 2505 units of killer cells whereas the control mouse cells produced 4635 units. It was possible that the reduced CTL production by cells from TCDD-treated mice could have been caused by a toxic destruction of the precursors of CTL. However, in the lower panel of the figure, we actually measured the frequency of precursors by limiting dilution and as can be seen, there was in fact a slight increase in the frequency of CTL precursors (CTLP). We used these frequency measurements to calculate the number of precursors that had been added to the tube culture system and determined the yield (units of CTL activity generated per CTLP). This figure was reduced by 60.7 % in this experiment in animals treated with 0.4 μg/kg TCDD.

Figure 3

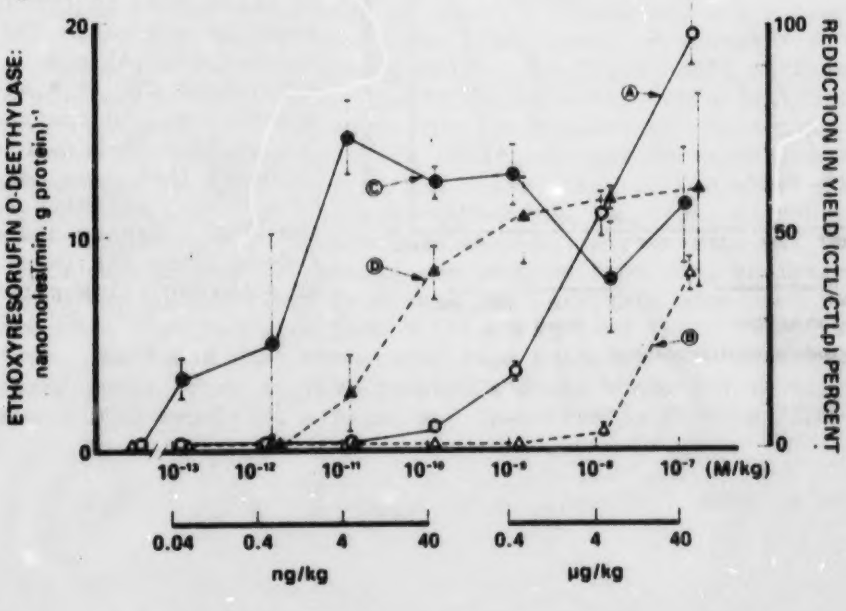

IRRADIATED STIMULATOR CELLS BEARING ALIEN H-2d ANTIGEN
1x10⁶
4.5 DAYS
37°C
NUMBER OF KILLER CELLS
CONTROL MICE
TCDD MICE (0.4μg/kg)
4635
2505
SUSPENSION OF LYMPH NODE LYMPHOCYTES
5000-100 PER WELL
IRRADIATED STIMULATIONS & HELPER CELLS
6 DAYS
37°C
FREQUENCY OF KILLER CELL PRECURSORS
1/292
1/211
YIELD
1.35
0.53
% SUPPRESSION
60.7%

The reduction of CTL generation per precursor after TCDD exposure could have been due either to a lack of helper T cells or to an increase in suppressor T cells. Since the thymus appeared to be a key target organ for TCDD toxicity, we conducted some experiments to directly determine if TCDD treatment generated suppressor cells in the thymus. The result from this experiment has been published elsewhere (18). Briefly, we found that thymus cells from mice given a total TCDD dose of 0.4, 0.04, or 0.004 μg/kg (4 ng/kg) became suppressive. Suppression could not be attributed to TCDD itself that might have been present in the thymus. We therefore concluded that TCDD treatment generates suppressor T cells and it would seem from Table II that these suppressor cells act selectively to inhibit only the CTL response.

Dose of TCDD required to suppress immune defences:

A striking result consistently obtained in the study of TCDD-induced immune suppression was the impairment of immunity by as little as 0.004 mcg/kg (18-20). Since TCDD also activated hepatic mixed function oxidase enzymes, such as ethoxyresorufin-O-deethylase (ERR), we compared the dose of TCDD required to produce liver alterations to that which suppressed immunity. As shown in figure 4, the immune response of the peripheral lymph nodes TCDD-

Figure 4



sensitive C57Bl/6 male mice was suppressed with 4 ng/kg (C,●) whereas 10-100 X this dose was needed to produce a measurable rise in hepatic enzyme activity (D,▲). Similar observations were obtained in studies of the immune response (A,○) and hepatic ERR activity (B,△) of DBA/2 mice which are genetically less susceptible to TCDD by virtue of having less TCDD receptor activity in its cells (6,7).

Selective suppression of CTL response by TCDD impairs resistance to infection: To determine if doses of TCDD that impaired only the CTL arm of the immune response also impaired immune defences in living animals (40 ng/kg - 4 µg/kg), we challenged treated and control (corn oil injected) mice with herpesvirus (19) type 2 i.p. The result of this study showed a significant increase in mortality in the TCDD-treated mice (table III). These data suggest that although mice exposed to ultra-low doses of TCDD appear healthy, they may suffer increased mortality if they are exposed to a type of pathogen such as herpesvirus where CTL appear to mediate immune resistance (14,21).

TABLE III

Effect of TCDD treatment on mortality following challenge with herpes virus II

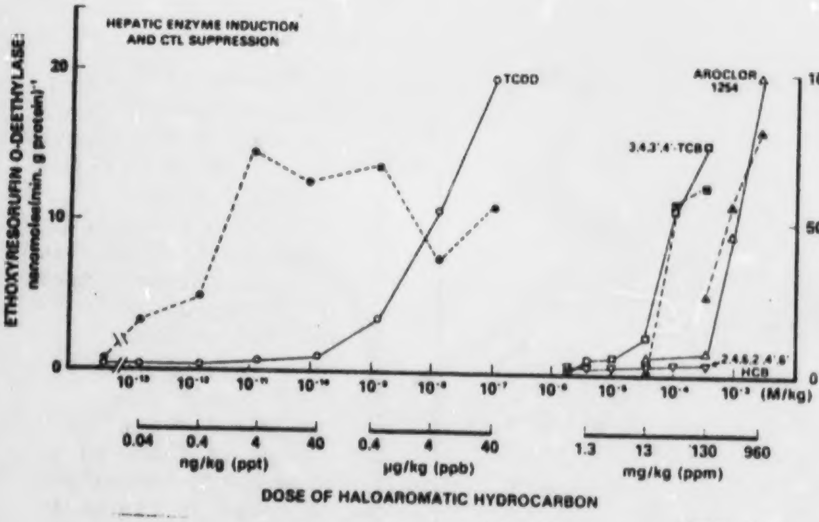
Treatment	*Proportion surviving*	*Significance*[a]
Vehicle control[b]	33/76 (43.4%)	—
40 ng/kg TCDD	15/65 (23.0%)	0.009
0.4 µg/kg TCDD	18/64 (28.6%)	0.044
4.0 µg/kg TCDD	18/63 (29.6%)	0.051

[a] P value in comparison to control was determined by Fisher's Exact test.

[b] Includes 16 untreated mice which had an identical survival to those receiving corn oil. All mice were male C57Bl/6J.

Other haloaromatic hydrocarbons that bind to TCDD receptor suppress immunity: A variety of compounds in addition to TCDD may produce chloracne and other TCDD-like lesions; these haloaromatic hydrocarbons include PCVs, PBBs, polychlorinated dibenzofurans, and polychlorinated naphthalenes (4). A common feature of active hydrocarbons appears to be an ability to bind to the TCDD receptor. Therefore, we tested a variety of these non-TCDD haloaromatics for their effect on hepatic mixed function oxidase enzymes and for an effect on the CTL response in vitro. As illustrated in figure 5, Arochlor 1254, a mixture of PCBs, and 3,3',4,4'-tetrachlorobiphenyl both of which bind to the TCDD receptor activated ERR enzymes (○,□,△) and suppressed CTL (●,■,▲) generation. In contrast, 2,2',4,4',6,6'-hexachlorobiphenyl that does not have a high affinity for the TCDD receptor did not induce liver ERR (▽) or suppress immunity (19).

Figure 5

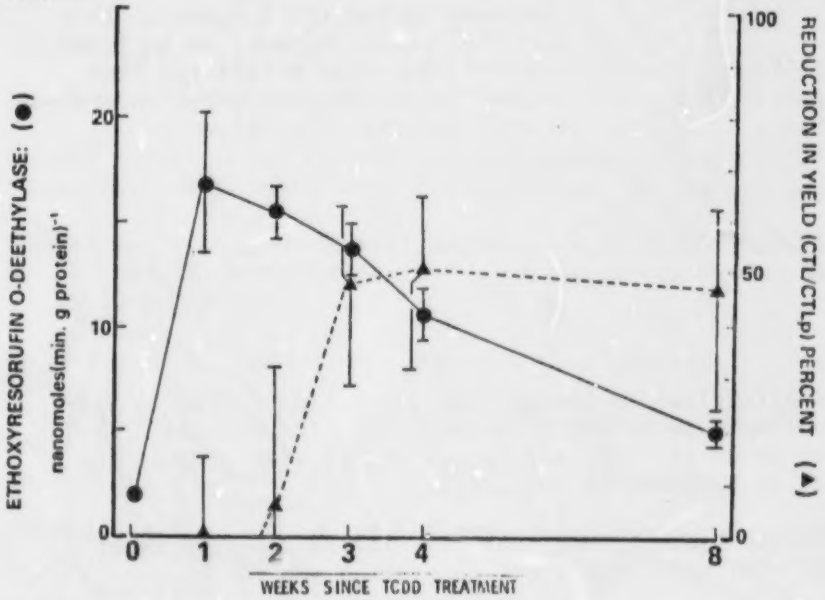


Imanishi et al (22) have reported that treatment of mice with the PCB mixture Kaneclor 500 significantly increases mortality following challenge with herpesvirus. Thus, PCBs may also produce the same defect in in vivo resistance to herpesvirus as we have shown with TCDD. The effect of the Kaneclor appears to occur with a molar dose ($3x10^{-6}$ M/kg) similar to the dose of 3,4,3',4'-TCB that is required to suppress the CTL response (19). Furthermore, Bekesi et al (23) have shown an in vitro impairment of T cell function in blood lymphocytes from humans exposed to PBBs. Data on the severity of viral infections in these subjects would be quite valuable in determining if humans incur a significant impairment of resistance as is seen in mice.

Development of immunosuppression following a single dose of TCDD:

Figure 6 illustrates the onset of TCDD toxicity as assessed by liver ERR. and CTL suppression. The mice were given a single injection of 0.4 μg/kg at time zero and were studied weekly thereafter for 4 weeks. It is readily apparent that hepatic ERR was rapidly induced and slowly declined in activity over an 8 week period. In contrast, immunosuppression was not evident until at least week 3 and persisted unchanged out to 8 weeks. Similar results were seen when 0.04 and 4 μg/kg TCDD were tested.

Figure 6



These data provided some clues concerning the mechanism of suppression by TCDD. The delay in the onset of suppression cannot be explained by a pharmacokinetic mechanism as the appearance of TCDD in thymus and other lymphoid organs following i.p. injection occurs during the first week. Precursors of T lymphocytes that mature in thymus and then migrate to spleen and lymph nodes include a short-lived population of suppressor cells with a half-life of a few weeks (24,25). This population is being continually replaced in the lymph nodes and spleen by new emigrants from the thymus. The kinetics by which TCDD generates increased suppressor T cell activity in the body is consistent with a stimulation of the short-lived suppressor T cell population in thymus. To test this idea, two types of experiments were conducted. First, chimeric mice were constructed in which the thymic stroma (which regulates T cell development in thymus) was derived from mice resistant to TCDD and the thymocytes were derived from susceptible strain mice, and vice versa. These animals were then injected with TCDD 0.004 μg/kg and their immune response studied in vitro. We found that thymocytes from animals DBA/2 resistant to this dose of TCDD became suppressive in TCDD-treated animals where the thymic epithelium was derived from a TCDD-sensitive strain, and that thymocytes from TCDD-susceptible C57Bl/6 mice failed to show increased suppressor cell activity when in the resistant DBA/2 strain host (20). The second type of study was conducted to enumerate the proportion of lymphocytes in PLN or spleen of TCDD-treated mice that possessed surface markers typical of short-lived suppressor T cells. In the mouse, these are called Lyt (lymphocyte) antigens and can be detected using an antibody to which a fluorescent label has been attached. We have used a cytofluograph which provides a sensitive and highly quantitative method for enumerating these cells, both in the mouse and in human blood, to test the effect of low dose TCDD treatment (4 ng/kg over 4 weeks) The result of 3 experiments in which murine lymph node cells were studied is illustrated in Table IV. The proportion of T cells bearing Lyt 2 was increased and further analysis indicted that the subset of Lyt 2 cells that also bear Lyt 1 was responsible for all of the increase in the Lyt 2 subset. This Lyt 1^+2^+ subset appears to include short-lived cells emigrating from thymus which act to promote suppressor T cell activity (24-26).

TABLE IV

Expt.	Lyt 1 only ("helper" T cells)	Ly 2 bearing
1	83.2 %[a]	118 %
2	85.0 %	121 %
3	105.0 %	135 %
	91.1 ± 7.0	125 ± 5.2[b]

a. TCDD as percent of result in vehicle-treated control.

b. Significant increase, $P < .05$

Summary and conclusions:

1. TCDD treatment of mice significantly impairs the generation of CTL in vitro and in vivo. This impairment is caused by stimulation of suppressor T cells, occurs at extremely low doses of TCDD, and is associated with a significant reduction in resistance to herpesvirus challenge.

2. Other types of haloaromatic hydrocarbons that can bind to the TCDD receptor protein in the cell can similarly impair CTL generation and resistance to herpesvirus challenge.

3. Although some strains of mice may be less susceptible to low dose TCDD, this resistance which is genetically determined by a gene or genes at the Ah locus is relative, and a sufficient dose of TCDD suppresses immune function of "resistant" animals.

4. Immunosuppression of mice with TCDD appears to increase the proportion of lymphocytes bearing the Lyt 2 marker that is found on suppressor T cells. Although a similar or analogous surface marker can be measured on suppressor T cells in human peripheral blood, it is not yet known if an increase in the number of suppressor cells is detectable in mouse blood. This information would be required before beginning a study of suppressor cells in haloaromatic hydrocarbon-exposed humans. Humans normally develop thymic involution during puberty so that the effects of TCDD and related compounds on the immune system might be expected to occur primarily in children and infants where the thymus is large and active.

5. The data obtained to date strongly implicates the epithelium of the thymus in the generation of suppressed immunity. Thymic epithelial cells elaborate a variety of regulatory molecules or thymic hormones (27) which control the immune system and assays now exist for some of these molecules. Measuring these thymic hormones in serum could provide a simpler and more sensitive test

for the immunotoxicity of TCDD and related haromatic hydrocarbons than measurement of suppressor cell number and activity in lymph nodes, spleen, or blood.

6. To date there has been little information concerning the possible effects of TCDD exposure on resistance to viral infections in humans apart from an increased frequency of complaints of colds (28) and it is possible that any immunological effects which occur following TCDD exposure may have minimal effect on resistance to most types of viruses. However, even small alterations in immunologic function indicate that the recipient has absorbed a sufficient amount of a haloaromatic hydrocarbon such as TCDD to incur a biological effect, and while the long term risk of such effects remains unknown at present their potential significance cannot be ignored.

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PARTITIONING OF MERCURY, LEAD AND CADMIUM IN AQUATIC SYSTEMS IN RELATION TO ACIDIFICATION

P.M. Stokes and L. Richman
Institute for Environmental Studies
and Department of Botany
University of Toronto
Toronto, Ontario M5S 1A1

1. Introduction

Acidification has the potential to influence the biogeochemical cycling of metals in aquatic systems. Recent concern over direct and indirect effects of acidic precipitation on surface waters has included consideration of potentially toxic trace metals. Some of these metals are "internally generated" from sediments, overburden on parent material, by increased weathering or dissolution from solid to dissolved phase as pH decreases. Al, Mn and Fe are in this category (1). Others are themselves trace contaminants which are loaded from external sources into the lake ecosystem and which may also increase in concentration as pH decreases (2). Cd and Pb are in this category. Zn would be in both categories.

Even if total dissolved metal concentration does not change, the chemical form or speciation of a number of metals in water or sediment is affected by pH. For example, Al changes solubility and dissolved species over the pH range 4-7, but Mn changes solubility but not speciation over the same range (3), while for Hg, there are speciation changes among methylated forms over this range (4).

Direct measurement of total or available metal in lakes of low alkalinity and depressed pH which are removed from point sources of metals generally reveals low levels in the water, although Al and Mn are exceptions in this respect. In the absence of comprehensive sets of data on precipitation chemistry, dissolved metals and biological uptake, a predictive approach has been used to anticipate which metals are expected to be of concern as lakes acidify. Campbell _et al._ (1983) (5) evaluated 18 metals on the basis of the five criteria shown in Table 1. It can be seen that while a large number of metals are mobilised by man's activities, a relatively smaller number show pH-related changes in solubility and/or speciation with pH. It is those metals which are of potential concern for aquatic biota and ultimately for man. The latter 2 columns in Table 1 address toxicity and bioaccumulation, respectively. Uncertainty increases from left to right on the table, and more data on biological effects are urgently needed.

2. _Biological effects of metals in acid lakes_

Direct toxicity to biota is a possibility as metals increase in concentration in acidic lakes. In a recent survey of 36 lakes in Ontario, Stokes _et al._ (6) determined levels of dissolved metals and by principal components analysis ordinated the lakes according to certain groups of chemical properties. Dissolved metals were negatively correlated with the component determined by pH and alkalinity. Excluding point sources, (in this case the Sudbury region), only Al and Zn were present in concentrations likely to cause acute toxicity (Table 2). Cd was determined in the water but was undetectable in all but 6 lakes. Al has of course been implicated already in a number of studies on fish toxicity in acid lakes (7,8), but to date no other metals have been shown to be related to changes in biota. But the effects of H+ per se cannot be separated from correlated increases in metals without an experimental approach in the field or laboratory.

More information is available on the metal content of biota, i.e. bioconcentration or bioaccumulation, as distinct from toxicity. The phenomenon of biological uptake is well known though poorly understood for metal contaminated systems (9,10) and a number of workers have discussed the use of living organisms as biological monitors of available metals (11). Because organisms are believed to provide a means of determining the Available portion of total metal, and because they generally contain higher concentrations than the surrounding water, they provide a convenient means of monitoring metals which may be difficult to determine or speciate in water. At the same time, there is concern for the consequences of biological uptake because of the potential for food chain transfer and toxicity to high level consumers such as man. Bioaccumulation has been noted in acid lakes for at least 7 of the metals (Table 1) in lakes unaffected by point source contamination.

The balance of this paper reviews some of the available information on mercury, lead and cadmium.

3.1. Mercury

Aside from gross contamination from industrial sources, mercury is found in trace quantities in aquatic systems remote from any point sources (12). Although concentrations of Hg in water may be at the parts-per-trillion level, recent observations have indicated that fish tissues accumulate mercury from these low levels, and that for particular species of fish the concentrations in fish are higher in lakes of lower pH. This has been documented for example in yearling perch in Ontario (13) (Figure 1), for pike in Sweden (14), for walleye in Ontario (15), for pumpkinseed in Ontario (16) and for walleye in Wisconsin (17). One of the most satisfactory hypotheses has been put forward by Jernelov and Johansson (18) who propose that while microbial methylation of Hg is relatively unaffected by pH, the final product is predominately monomethyl mercury (the bioavailable form) at low pH, in contrast to dimethyl mercury at higher pH (Figure 2). This hypothesis has not been supported by experimentation in many different laboratories, but it still provides the simplest explanation to date. Other factors influencing mercury cycling and uptake by biota include higher productivity, variously considered to decrease mercury bioaccumulation by fish (19,20) or to increase it (21), suspended material such as clay (22), and fish growth rates (23).

Recent studies on filamentous algae demonstrated a significant relationship between mercury in algae and mercury in fish (24,25) (Figures 3 and 4). In one study, the algal mercury was predominantly in the methyl form (24), supporting the contention that low pH increased the more available monomethyl form, since algae are not themselves known to methylate mercury. Recent data indicate the high levels of total mercury achieved by the algae (Table 3).

There are still technical problems with direct measurement of species of mercury in the low (2-20 ng/L) levels of total mercury typically found in water of acid-stressed lakes. However, a simple test of the hypothesis can be made in the laboratory. Earlier studies have measured direct uptake and food chain transfer of mercury in simple laboratory microcosms (26) but these workers were using much higher levels of mercury in water. We have designed experiments using methyl mercury in water without sediments, and measuring uptake at pH 6.8 and at 5.5 respectively, from initial concentrations of 50 and 100 ng/L . Organisms were selected on the basis of their occurrence in oligotrophic lakes over the selected pH range, their ease of handling and culturing in the laboratory and their acceptability as food.

After an extensive literature search, field sampling and laboratory trials, the amphipod Hyallela azteca was selected as primary consumer. It is an omniverous general scavenger in addition to being an important food source for many species of fish. It is found in acid-stressed lakes and in laboratory conditions shows mortality between pH 4 and 5 (27).

The selected food for Hyalella is Oocystis polymorpha;

as well the filamentous Mougeotia sp. isolated from an acidic lake will be used.

When direct uptake has been measured for both algae and for amphipods, and transfer from algae to amphipods via comsumption has been determined, experiments with yearling perch will be carried out to complete the series.

3.2. Lead and cadmium

Although there is far less information available for lead and cadmium in biota of acid-stressed lakes. at least two separate studies have shown a relationship between levels of these metals in fish and pH of lakes (28,17). No such relationship was found in the survey of metals in algae (6); indeed for cadmium in particular most samples had undetectable cadmium and although algal lead was generally quite high, it was not possible to determine a relationship with lead in fish. A mechanism underlying the observed pH relationship with Cd or Pb has not been hypothesised but is unlikely to parallel that proposed for Hg.

A similar series of experiments is planned for partitioning of Cd and Pb in the model food chain described for Hg. Again, water concentrations will be realistically low, which is in contrast to other laboratory studies of metal uptake.

4. Summary

Using controlled experimental conditions, the effect of pH on the uptake, accumulation and food chain transfer of

Hg, Cd and Pb from water containing very low levels of the respective metals, will be determined. These experiments are designed to follow up correlations observed in field studies of metals in biota in acidic and neutral softwater lakes.

5. References

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TABLE 1

METALS OF CONCERN IN RELATION TO ACIDIFICATION OF SURFACE WATERS

METAL	ATMOSPHERE ANTHROPOGENIC CONTROL	INCREASED TOTAL CONCENTRATION	SPECIATION CHANGE		TOXICITY		BIOACCUMULATION
			CALC.	OBS.	INH.	OBS. (IN ACID LAKES)	
AG	+				++		
AL		++	+	+	-	+	+
AS	+				+		
BE					-		
CD	+	+			++		+
CO	+			+	+		
CU	+		+		+		
HG	+	+	+	+	++		+
MN	+	++	+	+	-		+
MO							
NI	+				+		+
PB	+	+	+		+		+
SE	+				++		
SN					+		
TE							
TL					++		
V	+			+	++		
ZN	+	++		++	+		+

From Campbell et al., 1983. (Source, Reference 5)

TABLE 2

METALS	BACKGROUND[1] WATER LEVELS (PPB)	RANGE IN 1982 SURVEY STOKES ET AL.	TOXIC THRESHOLD (PPB)
ALUMINUM	30	46-372	100 (FISH)[2]
CADMIUM	0.07	1	0.2 (ZOOPLANKTON)[3]
COPPER	1.8	1-17	50 (ALGAE)[4]
LEAD	0.2	2-11	30 (ZOOPLANKTON)[5]
MANGANESE	5	5-276	-
NICKEL	0.3	2-6	30 (ZOOPLANKTON)[2]
ZINC	10	2-44	15 (ZOOPLANKTON)[3]

[1] FORSTNER AND WITMANN, 1981

[2] NRCC, 1981

[3] MARSHALL ET AL., 1981

[4] NRCC, 1979

[5] NRCC, 1978

(Source, Reference 6)

TABLE 3

TOTAL MERCURY IN ALGAE FROM SOFTWATER LAKES, 1983

LAKE	REGION	pH	TYPE OF GROWTH	TOTAL MERCURY µg g^{-1} DRY WEIGHT
Tantaré	Laurentide Park, P.Q.	5.0	Natural (on macrophytes)	3.7
Least	Chapleau, Ontario	5.6	Artificial substrate	3.55
Horton	Chapleau, Ontario	6.4	Natural substrate	0.02
Chub	Dorset, Ontario	5.8	Artificial substrate	0.15
Lake 223	ELA	5.0	Natural (on macrophyte)	0.90
			Artificial	0.38

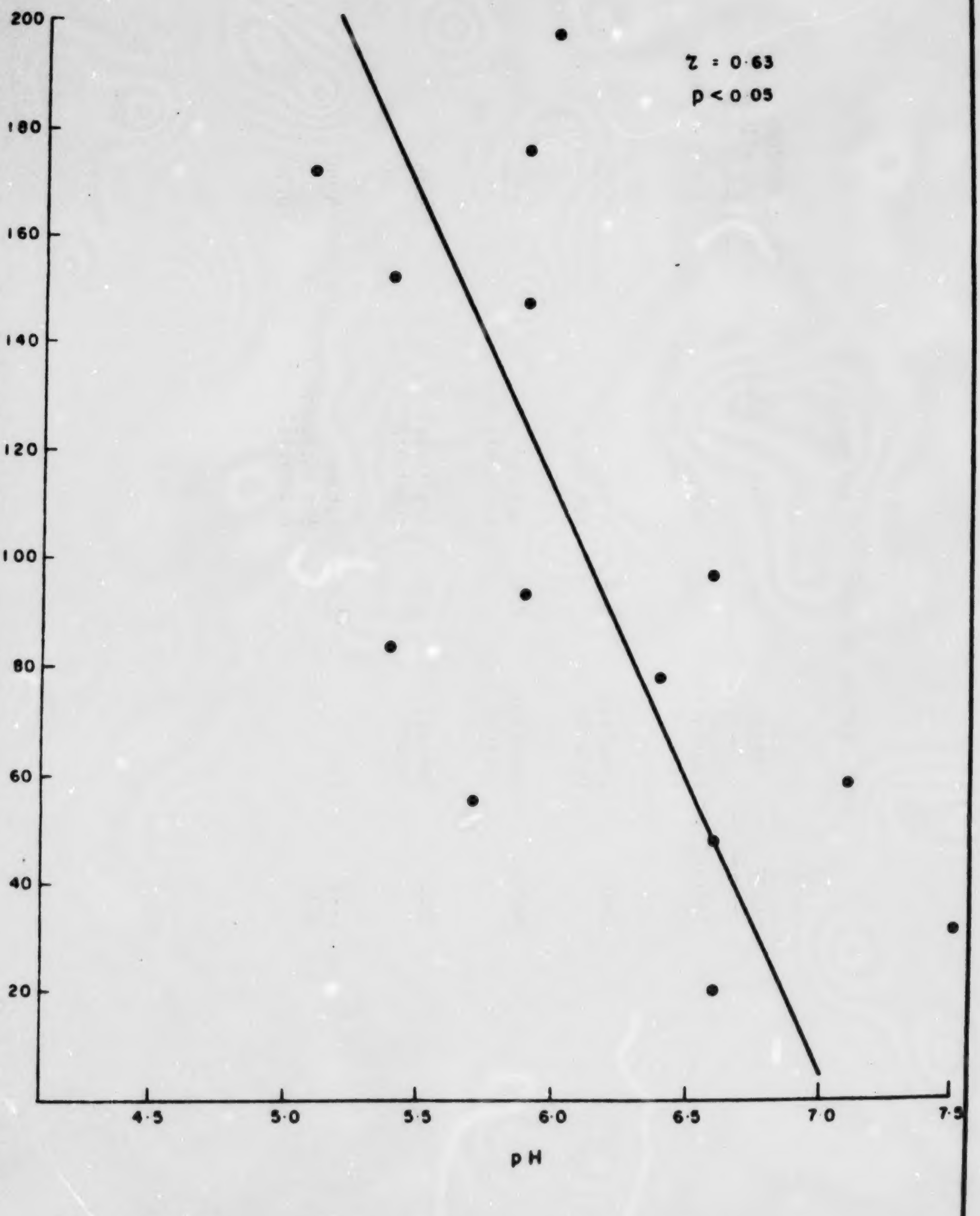


Figure 1: Mercury in yearling yellow perch and epilimnetic pH relationships. (Source, reference 13)

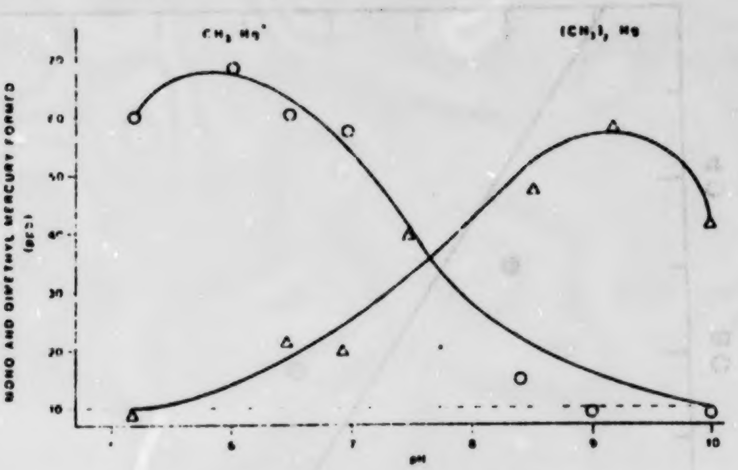


Figure 2: Formation of mono- and dimethyl mercury in organic sediments at different pH levels during 2 weeks, with a total mercury concentration of 100 ppm in substrate. (Source, reference 4)

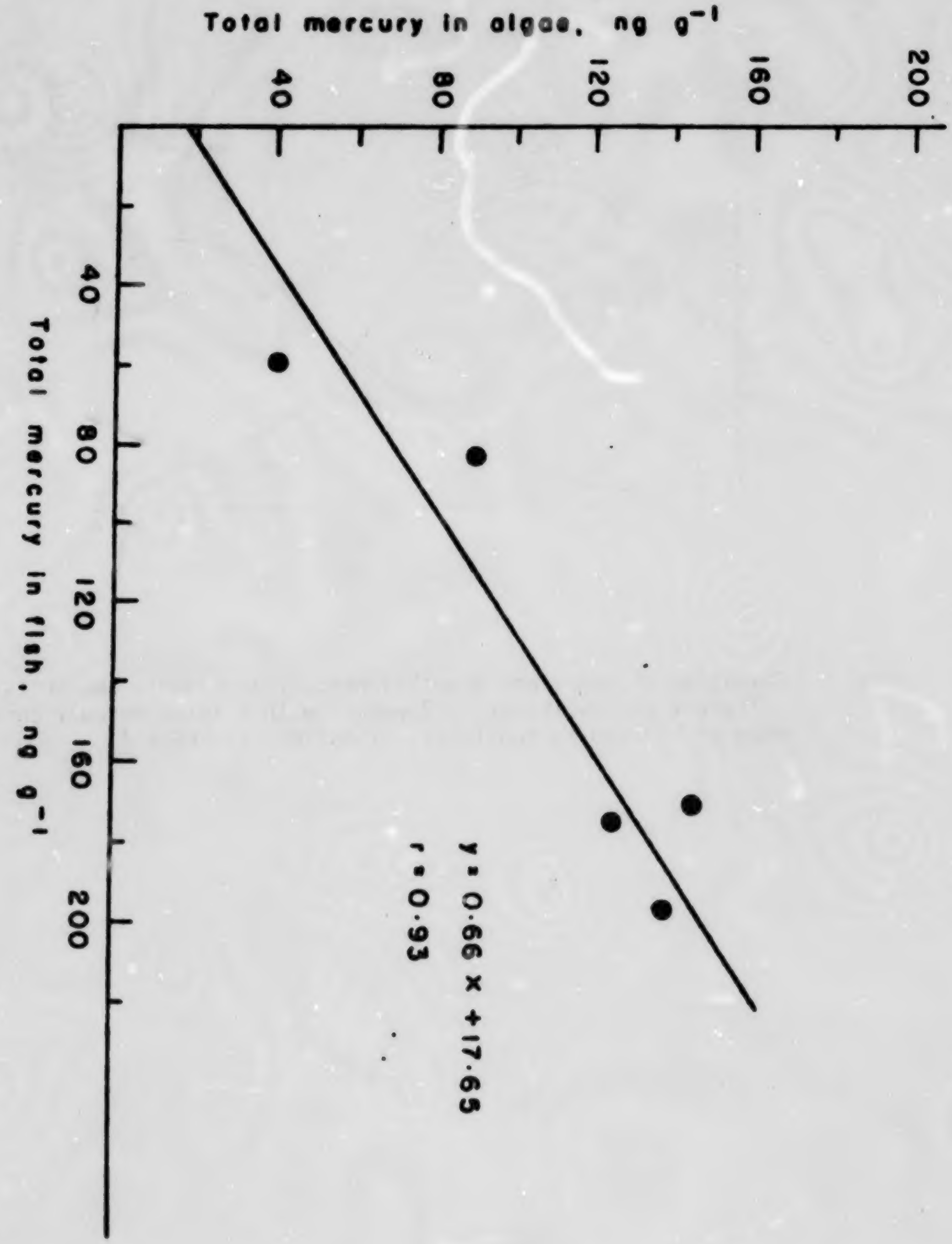


Figure 3: Relationship between mercury in yearling perch and mercury in algae, Dorset lakes, 1982. (Source, reference 24)

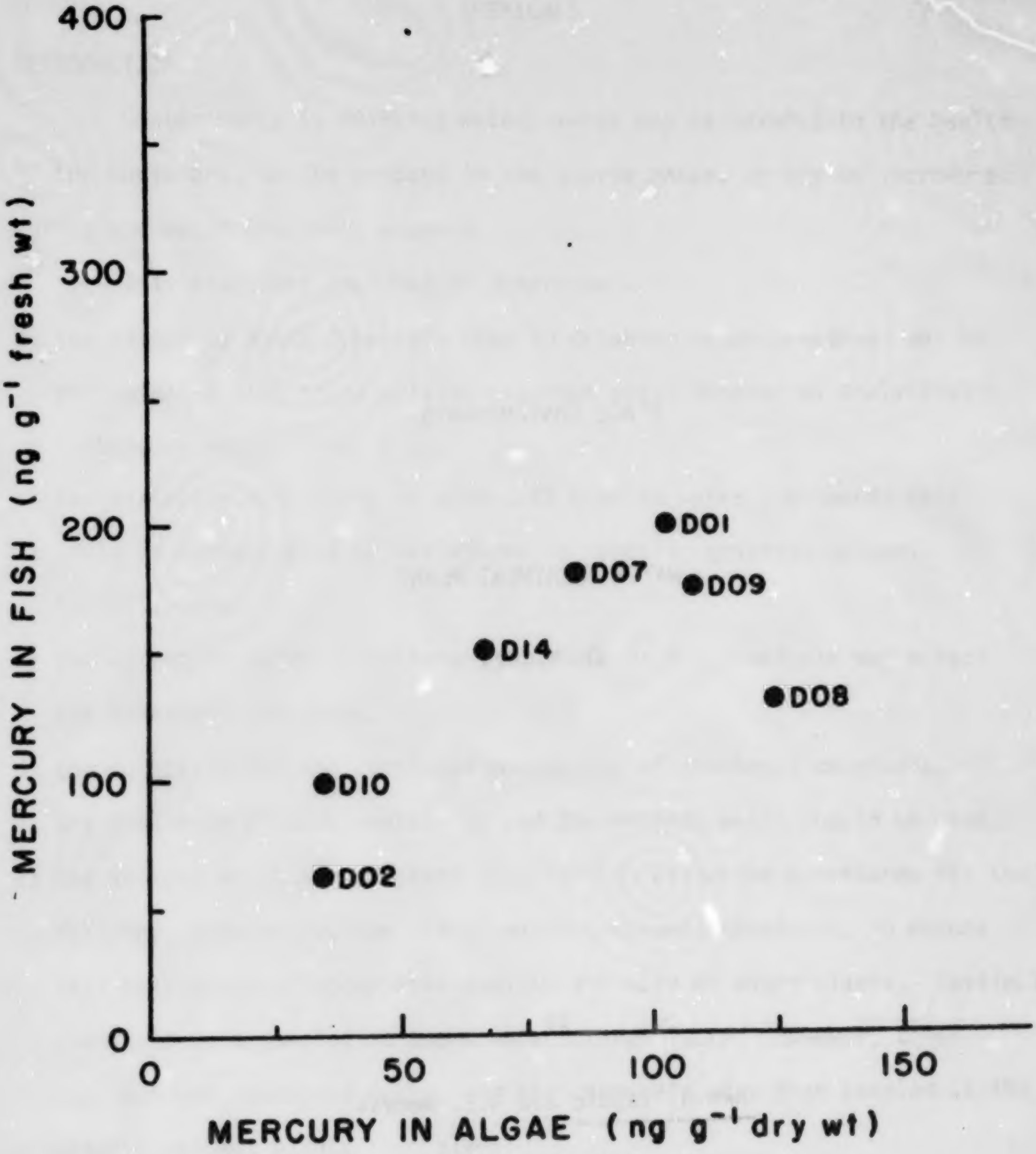


Figure 4: Relationship between mercury in yearling perch and mercury in algae, Dorset lakes, 1983. (Source, reference 25)

TRACE CONTAMINANTS
IN
WATER TREATMENT PLANT
CHEMICALS

by

Ann H. Vajdic and D.E. Wemyss
Water Technology Section
Ontario Ministry of the Environment
P.O. Box 213
Rexdale, Ontario
M9W 5L1

TRACE CONTAMINANTS IN WATER TREATMENT PLANT CHEMICALS

INTRODUCTION

Contaminants in drinking water, which may be harmful to the health of the consumers, may be present in the source water, or may be introduced during the water treatment process.

This study was designed to determine -

a) the extent to which chemicals used in drinking water treatment may be contaminated with trace metals, organics etc., insofar as analytical techniques would allow.

b) the variation in quality of chemicals used in water treatment; this could be demonstrated by variations in specific gravity, colour, turbidity etc.

c) the extent to which variations in quality of the chemicals may affect the treatment processes.

d) the necessity for the continued monitoring of treatment chemicals, the frequency of such monitoring and the methods which should be used.

e) the actions which are required to establish effective procedures for the delivery, examination and use of water treatment chemicals, to ensure that only those of acceptable quality are used at water plants. Initially, chemicals were sampled at the manufacturing plants. However, this approach was abandoned early, and the chemicals were then sampled at the water treatment plants themselves.

This type of sampling programme had the added advantages of -

a) detecting contamination that might occur during transportation to the water plants, due to improper handling and/or storage, or the use of non-dedicated carriers eg. tanker trucks previously used for the transportation of other chemicals.

b) the samples taken would automatically sample more frequently the products of the manufacturers supplying the largest number of plants, and thus the largest quantities of chemicals.

The programme consisted of the following stages -

a) the development of a list of the most frequently used chemicals employed in water treatment in Ontario

b) an examination of production processes, where appropriate

c) the physical analysis of each chemical, where appropriate, including specific gravity, pH, colour and turbidity

d) the analysis of each chemical for trace metals and organic compounds, where possible

e) the development of broad recommendations for future monitoring, and further actions

f) the development of a guide for plant supervisors and operators for assessing chemicals upon arrival at treatment plants, and ensuring the safe, efficient handling and storage of those chemicals. (Appendix 1).

SAMPLING

Samples were categorized, as shown in Table 1, into primary coagulants, treatment aids (coagulant aids, water conditioners, filtration aids etc.), raw and treated waters, sludges and fluoridation chemicals. Sludge and treatment plant chemical samples were taken in wide-mouthed, acid-washed plastic bottles. Water samples were taken in 1L glass bottles; for metals other than mercury, 1L glass bottles were used preserved with 2 mL of 50% nitric acid per litre; for mercury 1 mL of concentrated nitric acid and 0.5 mL drops of saturated potassium dichromate solution was added to samples taken in bacteriological sample bottles according to laboratory specifications. (1). The water plants at which the treatment chemicals were sampled are shown in Table 2.

The water plants were selected on the basis of the following criteria -

TABLE 1

WATER TREATMENT CHEMICAL CATEGORIES

PRIMARY COAGULANTS -	Aluminum sulphate - solid
	Aluminum sulphate - liquid
	Ferric chloride
	Sodium aluminate
TREATMENT AIDS -	Calcium oxide (unslaked lime)
	Sodium bicarbonate
	Sodium carbonate (soda ash)
	Sodium hydroxide
	Activated carbon
	Cat Floc T
WATER -	Raw, untreated water
	Finished, treated water
SLUDGE -	Usually a mixture of old sludge, from settling basins and new sludge, from settled backwashes
FLUORIDATION CHEMICALS -	Sodium silicofluoride
	Hydrofluorosilicic acid

TABLE 2

Water Plants Sampled for Treatment Chemicals

Plant	Location	Source of Supply	Capacity (1000m)^3
Stage 2			
Caldwell W.S.*	Verner	Veuve River	1.059
R.C. Harris WTP**	Toronto	Lake Ontario	1000.12
R.L. Clark WTP	Toronto	Lake Ontario	454.6
Stage 3			
Elgin Area W.S.	Yarmouth Twp.	Lake Erie	45.5
R.H. Neath WTP	Owen Sound	Georgian Bay	30.004
Sarnia City W.S.	Sarnia	Lake Huron	181.84
Goderich WTP	Goderich	Lake Huron	12.001
Port Elgin WTP	Port Elgin	Lake Huron	8.706
R.L. Clarke WTP	Toronto	Lake Ontario	454.6
Grand Bend Supply	Grand Bend	Lake Huron	168.2
Stage 4			
Easterly WP	Scarborough	Lake Ontario	454.6
Lindsay WTP	Lindsay	Scugog River	22.730
Kenora WTP	Kenora	Lake of the Woods	26.594
R.C. Harris WTP	Toronto	Lake Ontario	1000.12
Arnprior WTP	Arnprior	Madawaska River	15.456
Cornwall WTP	Cornwall	Lake St. Lawrence	100.012
Hawkesbury	Hawkesbury	Ottawa River	18.184
Ottawa WSS	Brittania	Ottawa River	247.302

*WS - water system

**WTP - water treatment plant

a) they received treatment chemicals from a good cross-section of the chemical manufacturers.

b) they had large flow rates. The greater the volume of water treated, the quicker the turn-over of chemicals and therefore the fresher the chemicals tested.

c) they were distributed as evenly as possible across the Regions and represented both lake and river supplies.

ANALYTICAL METHODS

A. Physical

Colour and turbidity were examined visually and measured photometrically with a Hach DR2 spectrophotometer and any gross foreign contamination was noted.

The pH was determined electrometrically, directly and in 1% solutions prepared in distilled water, on a Fisher Accumet pH meter. Specific gravity was determined by weighing on a Sauter balance, that had been checked for accuracy. Electron microscope studies were carried out using a Siemans 102 model, transmission electron microscope.

B. Chemical

Analysis for trace metals and organic compounds was carried out using standard preparatory techniques, followed by measurement by atomic absorption, emission spectography, gas chromatography and spectrophotometric colorimetry. Analyses were generally carried according to the methods prescribed in the "Outlines of Analytical Methods". (1). Some ad hoc modifications were required, due to the nature of some of the samples, along with the use of special standards.

Some types of sample could not be analysed because of severe interference or a highly corrosive effect on laboratory equipment. Other analysis produced results with very wide confidence limits, or of the "less than" variety, which were essentially qualitative. Chlorine was not able to be analysed for technical reasons, as was the case with many of the polyelectrolytes however, the former is generally used at dosages of less than 5 ppm and the latter at less than 1 ppm.

PROGRAMME

In the initial stages, plant chemicals and raw and treated waters and water plant sludges from various water plants, were analysed. Analysis for thirty trace metals, organics by solvent extraction/gas chromatography as well as for physical properties was completed. As the programme progressed, it became evident that some analyses were not going to be successful for some types of sample, and they were abandoned. For example, electron microscope analysis of primary coagulants was terminated because a suitable method could not be developed; solvent extraction and gas chromatography analysis was not done for the raw and treated water samples or the treatment aids because initial results had been consistently negative. Analysis for trace metals was changed from emission spectrography to atomic absorption to obtain more accurate results.

The programme was thus a dynamic one, with changes being made, as appropriate, when results were received in order to obtain the most accurate results. The analyses carried out in the programme are shown in Chart A.

RESULTS

In order to assess the effects of the various contaminants in drinking water chemicals on the treatment process and the quality of the finished water, it is most useful to discuss selected results collated from the whole programme. In this way, a total picture of quality variation in treatment chemicals both with respect to contamination levels and physical properties can be obtained.

CHART A

	Analyses	Primary Coagulants	Treatment Aids	WTP Raw/Treated Waters	WTP Sludge	Fluoridation Chemicals
STAGE 1	Trace Metals	List A1				
	Physical Properties	List B1				
	Solvent Extraction/ Gas Chromatography					List C
	Electron Microscope	List D				
STAGE 2	Trace Metals	List A2		List A2	List A2	
	Physical Properties	List B1				
	Solvent Extraction/ Gas Chromatography	List C		List C	List C	List C
	Electron Microscope	List D				
STAGE 3	Trace Metals	List A2	List A2	List A2	List A2	List A2
	Physical Properties	List B1				
	Solvent Extraction/ Gas Chromatography	List C			List C	List C
	Electron Microscope			List D		
	General Chemical			List E		
	Carbon Analyser	List F	List F	List F	List F	
	Mercury			List G		
	Loss on Ignition	List H2	List H2		List H2	
	Solvent Extraction/IR	List J	List J		List J	
STAGE 4	Trace Metals	List A2	List A2	List A2	List A2	
	Physical Properties	List B2				
	Solvent Extraction/ Gas Chromatography	List C				List C
	Electron Microscope					
	General Chemical			List E		
	Carbon Analyser	List F	List F	List F	List F	
	Mercury	List G	List G	List G		
	Loss on Ignition	List H1			List H2	
	Solvent Extraction/IR	List J	List J		List J	

List A

Trace Metals

- A2
  - A1
    - Cadmium
    - Chromium
    - Copper
    - Nickel
    - Lead
    - Manganese
    - Zinc
    - Cobalt
    - Tin
    - Molybdenum
  - Barium
  - Mercury
  - Iron
  - Silver
  - Aluminum
  - Arsenic
  - Beryllium
  - Bismuth
  - Boron
  - Zirconium
  - Calcium
  - Gallium
  - Germanium
  - Indium
  - Magnesium
  - Tellurium
  - Silicon
  - Antimony
  - Strontium
  - Titanium
  - Vanadium

List B

Physical Properties

- B2
  - B1
    - Specific Gravity
  - Turbidity
  - pH
  - % Alumina
  - % Free Acid
  - Colour/Turbidity

List C

Solvent Extraction/
Gas Chromatography

- 2-4 DNT
- 2-6 DNT
- TND
- Other N Compounds

List D

Electron Microscope

Asbestos Fibres &
Other Particles

List E

General Chemical

- pH
- Alkalinity
- Hardness
- Chlorine
- Iron
- Turbidity

List F

Carbon Analyser

Dissolved Carbon

List G

Mercury

Hg
Flameless Atomic Abs.

List H

Loss on Ignition

- H2
  - H1
    - % H_2O
  - % Loss on Ignition
  - Total Carbon

List J

Solvent Extraction

Methylene Chloride Extraction
Infra Red Spectrophotometry

TRACE METAL CONTAMINATION

Table 3 shows the recommended limits or maximum acceptable concentration (MAC) for selected trace metals from the Ontario Drinking Water Objectives (2); further, the table shows the concentration of metal that would have to be present in the drinking water chemicals to result in the MAC appearing in the finished drinking water, assuming a dose of 50 ppm, 25 ppm or 5 ppm of the chemical has been added for treatment. For example, for a chemical used at a dose of 50 ppm, 1000 ppm of arsenic would have to be present in that chemical to produce the MAC (0.05 ppm) in the finished water; at a dose of 25 ppm, then 2000 ppm of contaminating arsenic would be required and at 5 ppm, the required arsenic level would be 10,000 ppm. In the following discussion, the four most toxic metals will be considered. Since there was a good deal of interference with the analytical techniques, the accuracy of many of the results was equivocal, being of the "less than" variety or having wide confidence limits.

Some typical results of trace metal analysis in primary coagulants are shown in Table 4. As can be seen, one sample of liquid alum showed manganese, iron, titanium and vanadium levels considerably higher (more than five times) than the other samples; two samples showed chromium levels higher and one showed a lead level of 96 ppm as opposed to less than 1 ppm for the other two samples. The samples of solid alum showed similar trends, with sample 1 showing elevated metal levels. In primary coagulants the highest definitive level of arsenic recorded was 0.08 ppm, the highest for cadmium was 0.67 ppm, for lead 96 ppm and mercury 0.05 ppm; the equivalent highest recorded levels were less than 300 ppm, less than 80 ppm, less than 80 ppm and less than 300 ppm respectively. These levels may be plotted on a chart and compared to those levels (from Table 3), required to produce the MAC in the finished water. Such a plot is shown in Chart 1, assuming the coagulants are used at a dose of 50 ppm.

TABLE 3. RECOMMENDED LIMITS FOR METALS IN DRINKING WATER AND CONTAMINATION LEVELS REQUIRED IN TREATMENT PLANT CHEMICALS TO REACH THOSE LIMITS, ASSUMING THE SPECIFIED DOSAGES.

Metal	Recommended Limits	Concentrations of contaminant in treatment chemical that is required to donate the MAC at the following dosages		
	MAC*(mg/L)	50 ppm	25ppm	5 ppm
Arsenic	0.05	1,000	2,000	10,000
Barium	1.0	20,000	40,000	200,000
Boron	5.0	100,000	200,000	1,000,000
Cadmium	0.005	100	200	1,000
Lead	0.05	1,000	2,000	10,000
Mercury	0.001	20	40	200
Selenium	0.01	200	400	2,000
Silver	0.05	1,000	2,000	10,000
Uranium	0.02	400	800	4,000
Chromium	0.05	1,000	2,000	10,000

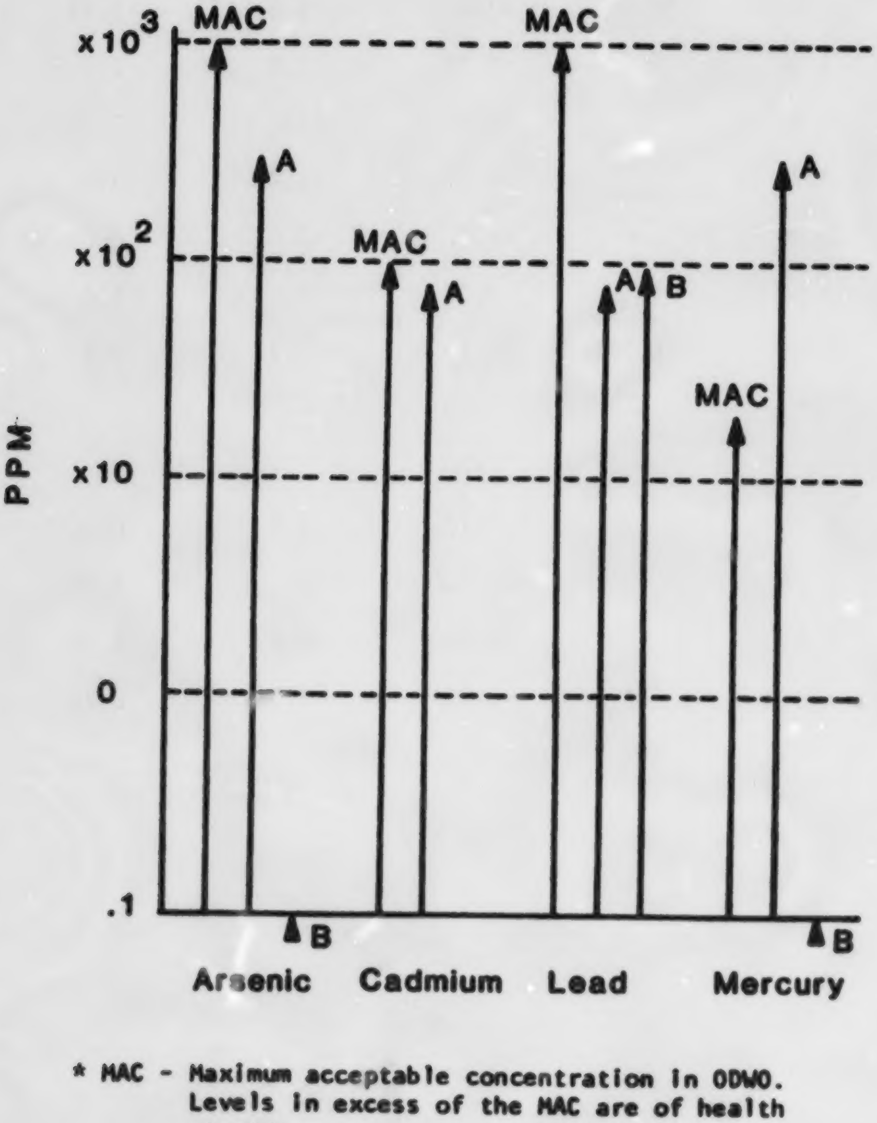
*MAC = maximum acceptable concentration, Ontario Drinking Water Objectives

TABLE 4. TYPICAL TRACE METAL RESULTS OF PRIMARY COAGULANTS.

	COAGULANT					
Trace Metal (ppm) as metal	Liquid Alum(1)	Liquid Alum(2)	Liquid Alum(3)	Solid Alum(1)	Solid Alum(2)	Ferric Chloride
Arsenic	-	-	0.08	-	-	<300
Beryllium	<0.03	-	<2	<0.03	<0.03	<10
Cadmium	<0.05	0.67	0.03	<0.05	<0.05	<10
Cobalt	<0.5	0.52	<0.2	<0.5	<0.5	10-30
Chromium	1.8	50.0	41.0	75.0	0.41	~10
Copper	2.14	0.67	<0.05	1.8	1.7	10-30
Iron	2.91	-	>2000	1530	0.82	-
Manganese	3.25	-	60-120	12.7	3.8	-
Mercury	0.05	-	<200	<0.01	0.03	0.05
Nickel	1.5	1.3	0.35	<0.5	1.0	10-30
Lead	0.88	0.25	96.0	13.1	<0.6	<10
Antimony	-*	-	<20	-	-	<30
Tin	-	-	<20	-	-	<30
Strontium	0.28	-	<60	2.4	0.18	<100
Titanium	2.15	-	600-2000	136	3.08	-
Vanadium	2.85	-	10-30	38.2	3.86	10-30
Zinc	1.6	2.6	<0.25	3.5	0.25	-

*no result available

CHART 1. COMPARISON OF THE HIGHEST LEVELS OF FOUR TOXIC METALS (ARSENIC, CADMIUM, LEAD and MERCURY) REPORTED IN PRIMARY COAGULANTS, WITH THE LEVELS REQUIRED TO PRODUCE THE MAC* IN THE FINISHED WATER, ASSUMING A DOSE OF 50ppm.



* MAC - Maximum acceptable concentration in ODWO. Levels in excess of the MAC are of health concern.

A - High Level

B - High Definitive Level

With the exception of mercury, none of the levels of metals found exceeded that required to produce the MAC in the finished water. With mercury, only the "less than" 300 ppm measurement, which is not considered reliable, is beyond the critical level.

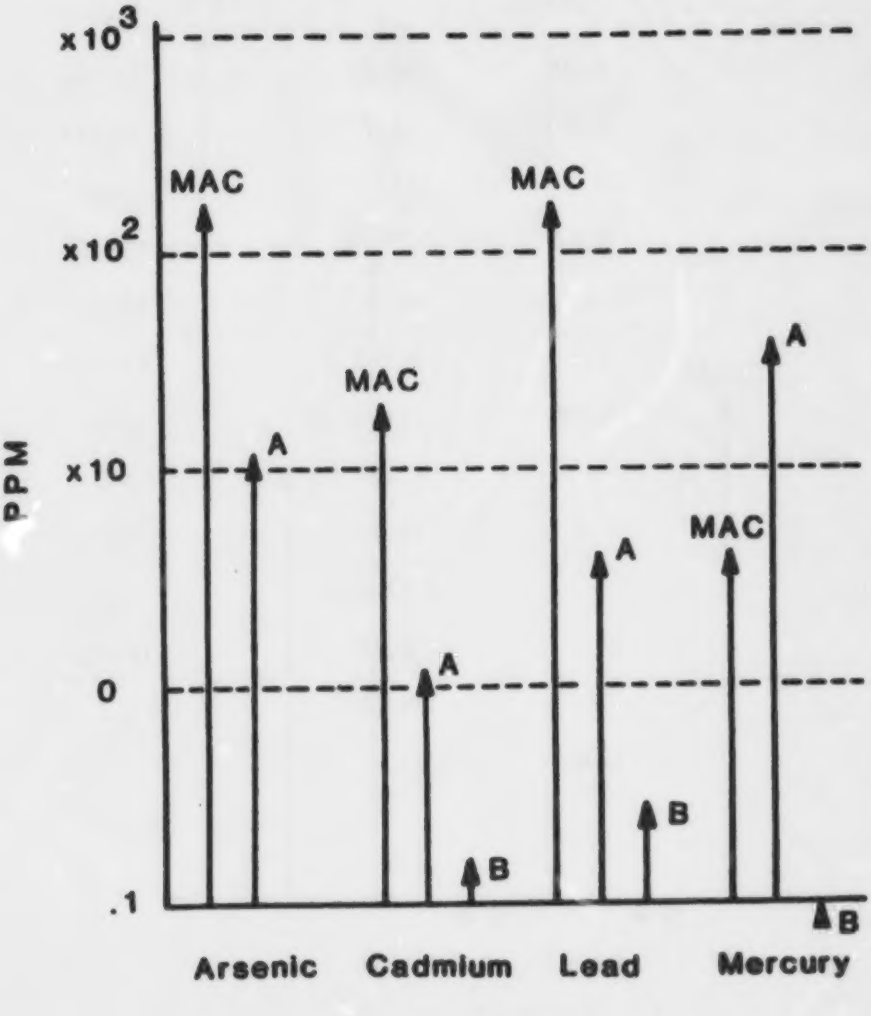
Table 5 shows typical metal analysis for the treatment aids; as can be seen, there was some variation in metal levels. Since these are generally used at lower dose rates than the primary coagulants, a plot similar to that developed for the primary coagulants was made, but using a dose rate of 25 ppm. The highest level found for arsenic was less than 120 ppm in unslaked lime and Cat-Floc T and there was no more accurately determined maximum. For cadmium, the highest level was less than 12 ppm in Cat Floc T and the highest definitive level, 1.6 ppm in unslaked lime. Lead showed corresponding levels of about 40 ppm in Cat Floc T and 2.9 ppm in unslaked lime, whereas mercury showed levels of less than 400 ppm in Cat Floc T and unslaked lime and no more accurately determined maximum; levels determined by atomic absorption spectrophotometry were <0.01 ppm. These levels are plotted in Chart 2. The fluoridation chemicals are usually used at a dose of about 5 ppm. Table 6 lists typical trace metal results for these chemicals. The highest level of arsenic recorded was less than 120 ppm in a sample of sodium silicofluoride, with a definitive level of 97 ppm being found in a sample of fluorosilicic acid.

For cadmium the highest level was less than 12 ppm in sodium silicofluoride with a level of 0.02 ppm in two samples of hydrofluorosilicic acid. Equivalent numbers for lead were less than 12 ppm in sodium silicofluoride and 0.09 ppm in hydrofluorosilicic acid, and for mercury, less than 400 ppm, in sodium silicofluoride with no accurately determined figure. As can be seen, however, from the plots in Chart 3, none of these levels except the questionable <400 ppm result for mercury would result in the MAC appearing

TABLE 5. TYPICAL TRACE METAL RESULTS OF VARIOUS TREATMENT AIDS.

	TREATMENT AID				
Trace Metal (ppm) as Metal	Activated Carbon	Unslaked Lime	Polymer Cat-Floc T	Sodium Bicarbonate	Sodium Hydroxide
Arsenic	<0.2	<120	<120	-	<100
Beryllium	<0.5	-	<12	<0.6	<0.5
Cadmium	0.1	1.6	<12	<0.05	<10
Cobalt	-	<1.0	-	<0.5	<1.0
Chromium	2.5	23.0	<12	0.12	tr <0.5
Copper	0.9	12.0	∿12	<0.6	0.5-1.0
Iron	540.0	∿4000	1200-4000	<0.36	10-20
Manganese	16.0	40-120	tr-<12	<0.6	tr-0.5
Mercury	-	<400	<400	<0.01	-
Nickel	3.1	2.6	tr-<12	<0.5	<3
Lead	<0.4	2.9	∿40	<0.6	<10
Antimony	-	<40	<400	-	<50
Tin	-	<40	<12	-	<10
Strontium	2.4	400-1200	<120	2.05	3-10
Titanium	8.0	12-40	<12	<1.25	<0.5
Vanadium	<2.0	tr-<12.0	<12	<1.25	-
Zinc	0.78	9.3	-	0.88	<10

CHART 2. COMPARISON OF THE HIGHEST LEVELS OF FOUR TOXIC METALS (ARSENIC, CADMIUM, LEAD and MERCURY) REPORTED IN TREATMENT AIDS, WITH THE LEVELS REQUIRED TO PRODUCE THE MAC* IN THE FINISHED WATER, ASSUMING A DOSE OF 25ppm.



* MAC - Maximum acceptable concentration in ODWO. Levels in excess of the MAC are of health concern.

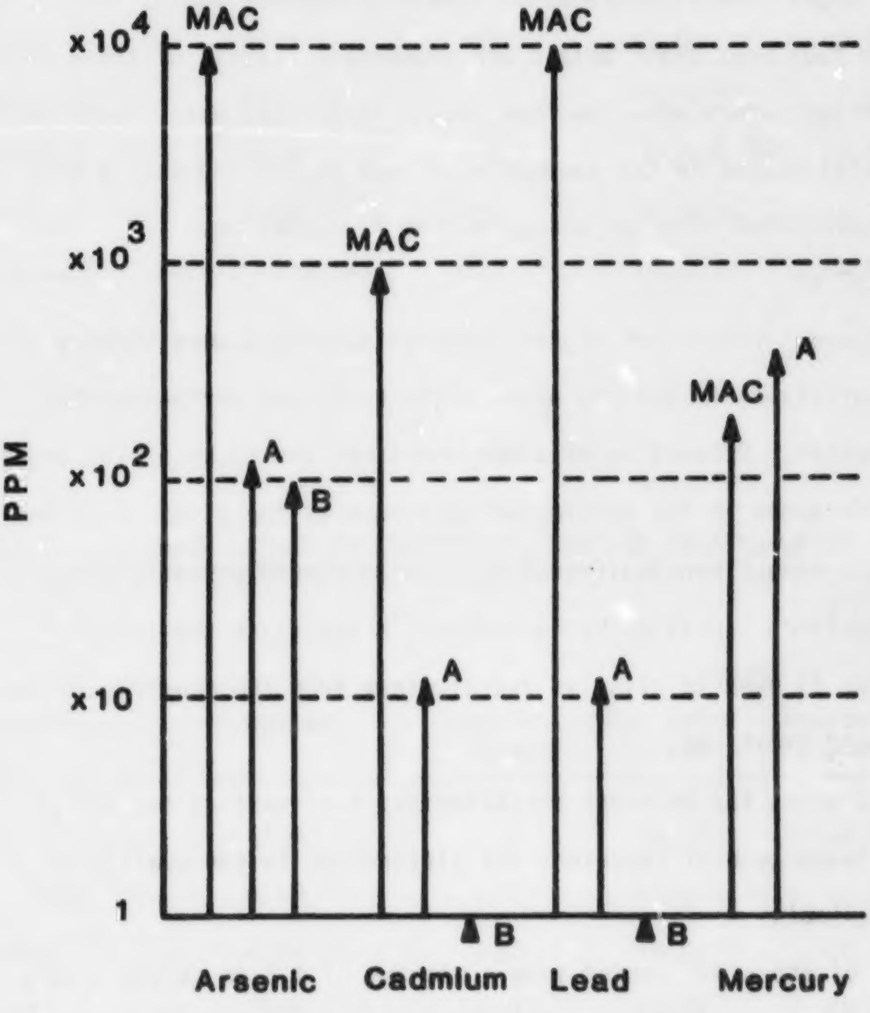
A - High Level

B - High Definitive Level

TABLE 6. TYPICAL TRACE METAL RESULTS OF FLUORIDATION CHEMICALS

	CHEMICAL		
Trace Metal (ppm) as Metal	Hydrofluorosilicic Acid 1	2	Sodium Silicofluoride
Arsenic	97.0	~0.8	<120
Beryllium	-	<0.27	<12
Cadmium	0.02	<0.27	<12
Cobalt	-	-	-
Chromium	0.34	~0.27	tr-<40
Copper	1.4	~2.7	tr-<12
Iron	24	>27	~40
Manganese	0.64	0.27-80	40-120
Mercury	-	<0.8	<400
Nickel	1.1	0.8-2.7	tr-<12
Lead	0.09	tr-<0.27	<12
Antimony	-	<0.27	<40
Tin	-	<0.27	<40
Strontium	-	0.27-0.8	<12
Titanium	-	8.27	tr-<12
Vanadium	-	~0.27	<12
Zinc	0.4	-	-

CHART 3. COMPARISON OF THE HIGHEST LEVELS OF FOUR TOXIC METALS (ARSENIC, CADMIUM, LEAD and MERCURY) REPORTED IN FLUORIDATION CHEMICALS, WITH THE LEVELS REQUIRED TO PRODUCE THE MAC* IN THE FINISHED WATER, ASSUMING A DOSE OF 5ppm.



* MAC - Maximum acceptable concentration in ODWO. Levels in excess of the MAC are of health concern.

A - High Level

B - High Definitive Level

in the finished water if the chemicals were used at their usual dose rate.

As can be seen, the levels of these toxic metals found contaminating water treatment chemicals, are, for the most part, significantly below the levels which would result in a health concern in the finished water, even if used at abnormally high dose rates. Tables 7a, b, c and d, present the results of analysis of coagulant chemicals, raw and treated waters and water plant sludges from selected water treatment plants; again, the results of the four most toxic metals are presented. Levels of these metals in raw and treated waters were low, and levels in treated waters were below the MAC's. Metal levels in the sludges were much higher indicating that metals are precipitated into the sludge during flocculation.

PHYSICAL PROPERTIES

The physical properties of the primary coagulants were studied to determine variations in quality which might have some influence upon treatment processes. Properties of alums sometimes varied by a wide degree, due to the differences in the manufacturing processes for alum. Alum may be produced by a direct process (reacting sulphuric acid directly with bauxite in lead-lined tanks) or by an indirect process; in the latter, sodium hydroxide is used to dissolve the aluminum from the bauxite, followed by sulphuric acid treatment.

Table 8 shows the physical characteristics of various samples of liquid alum, chosen to best represent the differences in the quality of liquid alum available.

The pH of the alums varied from a minimum of 0.5 in sample 4 to a maximum of 2.5 in sample 6; the free acid occurred at a high of 1.23% in sample 4 and free alumina occurred at a high of 0.28% in sample 6.

Differences in the pH of alums could potentially affect the functioning of the chemical in treatment. As shown in Table 9, water with a moderate buffering capacity (100 ppm alkalinity) suffers a maximum pH reduction of

TABLE 7a. ARSENIC LEVELS IN COAGULANTS, RAW AND TREATED WATER AND WATER PLANT SLUDGES FROM SELECTED WATER TREATMENT PLANTS IN ONTARIO

Location	Coagulant	Arsenic in Coagulant (ppm)	Raw Water (ppm)	Treated Water (ppm)	Sludge (ppm)
R.C. Harris	Alum	<20	<0.05	<0.05	0.88
R.L. Clark	Alum	<20	<0.05	<0.05	0.73
Lindsay	Alum	<20	<0.05	<0.05	0.32
Kenora	Alum	<20	<0.05	<0.05	0.37
Owen Sound	Alum	<30	<0.0025	<0.0025	0.26
Goderich	Alum	<30	0.004	<0.0025	Int*
Sarnia	Alum	<30	<0.0025	<0.0025	Int*
Grand Bend	Ferric chloride	<300	<0.003	<0.0026	Int*

*Int = interference with analytical method

TABLE 7b. CADMIUM LEVELS IN COAGULANTS, RAW AND TREATED WATER AND WATER PLANT SLUDGES FROM SELECTED WATER TREATMENT PLANTS IN ONTARIO

Location	Coagulant	Cadmium in Coagulant (ppm)	Raw Water (ppm)	Treated Water (ppm)	Sludge (ppm)
R.C. Harris	Alum	<20	<0.01	<0.01	0.19
R.L. Clark	Alum	<20	<0.01	<0.01	<0.02
Lindsay	Alum	<20	<0.01	<0.01	<0.02
Kenora	Alum	<20	<0.01	<0.01	<0.02
Owen Sound	Alum	<10	<0.0005	<0.0005	0.51
Goderich	Alum	<10	<0.0005	<0.0005	<0.02
Sarnia	Alum	<10	<0.0005	<0.0005	0.1
Grand Bend	Ferric chloride	<10	0.0005	0.0005	0.6

TABLE 7c. LEAD LEVELS IN COAGULANTS, RAW AND TREATED WATER AND WATER PLANT SLUDGES FROM SELECTED WATER TREATMENT PLANTS IN ONTARIO

		Lead in			
Location	Coagulant	Coagulant (ppm)	Raw Water (ppm)	Treated Water (ppm)	Sludge (ppm)
R.C. Harris	Alum	<10	<0.05	<0.05	<0.4
R.L. Clark	Alum	<20	<0.05	<0.05	1.4
Lindsay	Alum	<20	<0.05	<0.05	<0.4
Kenora	Alum	<20	<0.05	<0.05	<0.4
Owen Sound	Alum	<10	0.004	0.007	<0.4
Goderich	Alum	<10	0.0095	0.006	5.6
Sarnia	Alum	<10	<0.0025	<0.0025	<0.4
Grand Bend	Ferric chloride	<10	0.007	0.009	14.7

TABLE 7d. MERCURY LEVELS IN COAGULANTS, RAW AND TREATED WATER AND WATER PLANT SLUDGES FROM SELECTED WATER TREATMENT PLANTS IN ONTARIO

		Mercury in			
Location	Coagulant	Coagulant (ppm)	Raw Water (ppm)	Treated Water (ppm)	Sludge (ppm)
R.C. Harris	Alum	<200	<0.00008	<0.00008	-
R.L. Clark	Alum	<200	0.00005	0.00005	-
Lindsay	Alum	<200	<0.00003	<0.00003	-
Kenora	Alum	<200	0.00005	0.00007	-
Owen Sound	Alum	<100	<0.00003	<0.00003	-
Goderich	Alum	<100	<0.00004	<0.00004	-
Sarnia	Alum	<100	<0.00004	<0.00004	-
Grand Bend	Ferric chloride	<300	<0.00004	<0.00004	-

TABLE 8. PHYSICAL CHARACTERISTICS OF LIQUID ALUMS DELIVERED TO SELECTED WATER TREATMENT PLANTS

Manufacturer/Location of Liquid Alums	Straight pH	Spec. Gravity @ 20°C	Colour/Turb Pt/Co units	Turbidity FTU	% Free Acid	% Free Alumina
1. Border Emo	1.10	1.329	25 clear beige	10	0.25	--
2. Border Kenora New	0.80	1.306	25 clear beige	10	0.85	--
3. Border Kenora Old	0.61	1.326	40 clear beige	20	1.19	--
4. Border Kenora	0.5	1.325	50 clear beige	20	1.23	--
5. Border Rainy River	2.18	1.320	50 clear beige	10	--	0.08
6. Alcan Kingston	2.50	1.325	50 clear beige	20	--	0.28
7. Allied Deseronto	2.25	1.339	210 green/blue, opal.	55	--	0.13
8. Allied Thorold	2.20	1.334	220 green/blue,clear	70	--	0.09
9. Allied Niagara Falls	2.15	1.332	290 green/blue,turbid	70	--	0.07
10. Allied Port Elgin	1.50	1.333	>500 green/blue, very turbid	225	--	0.01
11. Allied Deseronto	2.25	1.328	290 green/blue,turbid	65	--	0.17
12. Allied Hawkesbury	2.15	1.330	140 green/blue, opal	50	--	0.13

TABLE 9. pH OF 1% OF VARIOUS ALUMS AND REDUCTION OF pH AND ALKALINITY CAUSED BY THEIR ADDITION TO DIFFERENT WATERS

Sample	pH of 1% w/v soln.	Alkalinity Reduction per ppm Alum	Initial Reduction of pH	Reduction of pH after 15 min.	Type of Water & Alum Feed
1	3.55	0.45	0.75	0.30	100 ppm alk. 40 ppm alum
2	2.85	0.50	0.85	0.40	100 ppm alk. 40 ppm alum
3	2.7	0.50	1.45	0.90	20 ppm alk. 20 ppm alum

only 0.4 after 15 minutes, even with a low pH alum used at 40 ppm. However, water of a lower buffering capacity (20 ppm alkalinity) showed a substantial loss of 0.9 pH units in 15 minutes, with only 20 ppm of alum. In such waters, the use of a low pH alum could result in a reduction of pH and alkalinity in the water sufficient to impede floc formation.

Because the presence of turbidity can affect the measurement of colour, the turbidity of a sample must be considered when colour is measured. Colour, however does not affect the turbidity measurement when measured on a Hach DR2 spectrophotometer. The variation in colour/turbidity measurement was large, varying from 25 Pt/Co units in samples 1 and 2, to greater than 500 Pt/Co units in sample 10. Alums produced by the direct process were always blue to blue-green in colour and had an opalescence which varied from slight to very heavy, leading to higher colour/turbidity readings than in the beige alums produced by the indirect process. The former alums tended to have higher turbidity readings also, and contained higher levels of the trace metals. The alum, sample 10, with the turbidity of 225 Ftu should not have been accepted for water treatment purposes.

The specific gravity may be the most important physical parameter examined, since it indicates the strength of the liquid alums. Specific gravities varied from 1.306 for sample 2 to 1.339 for sample 11; a specific gravity of 1.335 yields the rated 48.5% w/w concentration that is quoted in the manufacturers specifications. Differences in specific gravity could affect the treatment process. Thus if the same dilution of alum is made, regardless of the specific gravity of the batch the concentration of the solution of the alum could vary greatly. As shown in Table 10 using the regular dilution figure for obtaining a 1% w/v solution, alum of specific gravity 1.306 resulted in a concentration of 9 mg/mL; alum of specific gravity 1.339, when diluted in the same manner, yielded a concentration of 10.15 mg/mL. This difference in concentration could well affect the

TABLE 10. Specific Gravity and Concentration of 1% Solutions of Various Alums

Manufacturer/Location	Specific Gravity	Concentration of 1% w/v solution
Border Chemical (Kenora (New))	1.306	9.0
Allied Chemical (Deseronto)	1.339	10.15
Allied Chemical (Oakville)	1.335	10.02
Allied Chemical (Alexandria)	1.335	10.02

flocculation process.

It is possible that some plant treatment problems have resulted from the use of a new batch of alum with a different pH or specific gravity from that used previously.

Since no methods could be developed to analyse treatment chemicals for asbestos and related fibres, it could not be determined whether chemicals could be a significant source of such material.

Investigations at the water treatment plants revealed that quality deterioration of chemicals can result from improper storage and handling practices. For example, open bin or open bag storage of lime allows moisture and carbon dioxide to produce a build-up of insoluble calcium carbonate; this reduces the potency of the chemical until it may become quite ineffective for its intended purpose.

ORGANIC ANALYSIS
SOLVENT EXTRACTION/GAS CHROMATOGRAPHY

Dinitro toluene was present at low levels in some samples of hydrofluorosilicic acid, the highest level being 2.4 ppm; this compound and other related compounds were recovered from one sample of water plant sludge (R.L. Clark in Toronto), but at levels of less than 0.5 ppm.

Low levels of organic materials were shown to be present by DOC analysis in the primary coagulants, but solvent extraction followed by infra-red spectroscopy was not successful. Results are shown in Table 11. Some treatment aids (unslaked lime, sodium silicofluoride and sodium bicarbonate) showed some unidentifiable organics by the infra-red analysis technique.

Solvent extraction and infra-red analysis on water plant sludges revealed a large number of broad bands in the spectrogram, but no individual organics could be identified. High levels of organics in sludges were

TABLE 11. INFRA RED SCANS AND DOC LEVELS IN ALUM FROM SELECTED WATER TREATMENT PLANTS

Water Plant	DOC (ppm)*	Infra Red
Owen Sound	3.7	NA*
Goderich	4.2	NA
Sarnia	5.0	NA
Elgin Area WS	3.7	NA
R.C. Harris	2.7	NA
Lindsay	2.9	NA
Kenora	1.8	NA
R.L. Clark	2.1	NA
Port Elgin	3.5	NA

*no organics extractable, or sample too corrosive.

also revealed after drying the sludges to constant weight, and then extracting an aliquot with methylene chloride; the methylene chloride extracts were then dried to constant weight and the weight of extracted organics recorded. The weight of extractable organic material is shown in Table 12. The levels of such material ranged from 4 ppm (mg/Kg dried weight of sludge), in the sludge from Kenora, to 11000 ppm at Toronto's Easterly plant. It is interesting that increasing levels of organic extractables were found in the sludges when progressing from Owen Sound to Goderich, Grand Bend and Sarnia southward along the Lake Huron shoreline. A similar trend occurred moving from the Elgin Area System on Lake Erie through to Lake Ontario and Toronto's R.C. Harris and Easterly plants. This corresponds fairly well with increasing urbanization and industrialization of the lakes adjacent to these areas. Kenora would be regarded as non-industrialized.

RECOMMENDATIONS

1. Quality control checks should be carried out on chemicals upon receipt at water treatment plants; if possible, especially for primary coagulants, these checks should include simple tests at the plant, such as pH, colour and colour/turbidity. Manufacturers specifications should conform to AWWA specifications for the chemicals, or such specifications as the Ministry of the Environment may introduce.
2. Unless quality control data and/or in-plant testing indicate an increase in the number of abnormal lots of chemicals of deteriorating quality, monitoring of chemicals need only be undertaken every 5 years.
3. Chemicals must be handled, stored and used correctly at water treatment plants. The Training and Certification Section of the Ministry of the Environment should be requested to provide instruction in the necessary techniques for use and analysis.
4. Results of quality control checks of treatment chemicals must be kept

TABLE 12. INFRA-RED SCANS AND TOTAL EXTRACTABLE ORGANICS IN WATER PLANT SLUDGES

Water Plant	Extractables (ppm)	Infra Red
Owen Sound	10	+*
Goderich	33	+
Grand Bend	360	+
Sarnia	4000	+
Elgin Area W.S.	790	+
R.C. Harris	1780	+
Easterly	11000	+
Kenora	4	+

*organics detectable but not identifiable at the water treatment plants for a period of at least 5 years; duplicate records should be forwarded to a central file at the Ministry of the Environment.

5. Research into the development of better analytical techniques for the examination of water treatment plant chemicals should be encouraged; this particularly applies to the detection and identification of organic contaminants.

REFERENCES

1. Outlines of Analytical Methods. Ontario Ministry of the Environment, LSB, 1981.
2. Ontario Drinking Water Objectives, MOE - in print.

APPENDIX 1

HANDLING, STORAGE AND ON-SITE TESTING OF CHEMICALS USED IN THE TREATMENT OF DRINKING WATER

The information given in this Appendix is intended as a guide for the water plant operator or supervisor. Included with details regarding the recommended handling and storage procedures, are recommendations for testing procedures to be carried out on chemicals upon receipt at the water plant, along with the appropriate AWWA Standard number. When ordering treatment chemicals, a request should be made that they conform to AWWA standards. Manufacturers should provide results of the testing for conformity with the specifications. Water plants should occasionally submit samples of chemicals received, for spot checking of specifications, to the Ministry of the Environment laboratories. Chemicals used in water treatment should contain no substances in quantities capable of causing injurious effects upon the health of those consuming the water to which the chemical has been added.

1. PRIMARY COAGULANTS

A) ALUMINUM SULPHATE ($Al_2(SO_4)_3$). AWWA Standard B403-70.

Aluminum sulphate (liquid or solid) is used for coagulation.

1. LIQUID

Storage - Liquid alum should be stored in lined steel, stainless steel, lead, rubber or plastic containers. Liquid alum should be shipped and stored at temperatures above -13.3°C; below this temperature the alum will crystallize out. Although it appears to redissolve, if warmed, the alum will never subsequently remain permanently in solution and its suitability for treatment will be compromised.

Precautions - Liquid alum is of low pH and corrosive; it is irritating to the skin; handling requires caution and the use of protective clothing and corrosion-resistant equipment.

Testing - The following visual and analytical tests should be carried out on liquid alums upon delivery at the plant.

a) Colour - alum should be beige or blue/green in colour; Alums which are bluish or bluish-green in colour are probably manufactured by the direct process; this process tends to produce alums with a higher trace metal content. Colour should be measured with a Hach DR2 spectrophotometer. The reading for colour using this instrument, is actually colour/turbidity since the latter interferes with the measurement of the former.

b) Turbidity - liquid alum should be sufficiently clear to enable flow measuring devices to be read without difficulty. Alums with turbidity, measured on a Hach DR2 spectrophotometer, exceeding 200 Ftu should be regarded as unsatisfactory.

c) pH and pH of 1% solution - excessively low pH in liquid alum could cause treatment problems, especially in low alkalinity waters; the pH of a 1% solution of it, should thus be measured to determine if it is significantly different from previous shipments.

Specifications. Manufacturers specifications should include results of the following tests; the AWWA specifications are given in parenthesis:

Available water soluble aluminum as Al (not less than 4.25%)
Available water soluble aluminum as Al_2O_3 (not less than 8%)
Excess water soluble Al_2O_3 (at least 0.025%)
Excess water soluble aluminum (at least 0.013%)
Total water soluble iron as Fe_2O_3 (no more than 0.35% based on 8% Al_2O_3).
Insoluble and Suspended Material (not to exceed 0.2%)

2. SOLID

Storage - Solid alum should be stored in a dry area, protected from moisture in sealed containers

Precautions - Handling of solid alum requires caution and the use of dust masks

Testing:

a) The size of solid alum is important whether it is to be dissolved or fed with a dry feeding machine. Sizing may be done with sieves, in which case 100% should pass through a 3" ring and 75% be retained on a 1/2" sieve (US standard Z23.1 - 1961).

Specifications. Manufacturers specifications should include results of the following tests; the AWWA specifications are given in parenthesis:

Available water soluble aluminum as Al (not less than 9%)

Available water soluble aluminum as Al_2O_3 (not less than 17%)

Excess water soluble Al_2O_3 (at least 0.05%)

Excess water soluble aluminum (at least 0.026%)

Total water soluble iron as Fe_2O_3 (no more than 0.75% based on 17% Al_2O_3)

Insoluble and suspended material (not to exceed 0.5%)

B) LIQUID FERRIC CHLORIDE ($FeCl_3$)

There are no AWWA Standards for ferric chloride; it is used as a coagulant.

Storage - Liquid ferric chloride should be stored in lined steel, stainless steel or plastic or rubber containers; it is normally supplied as a 35-45% solution.

Precautions - Liquid ferric chloride is stain-producing, corrosive and irritating to eyes, lungs and skin. Protective clothing and corrosion-proof equipment is necessary during handling.

Testing -

a) Ferric chloride from new suppliers should be analysed for heavy metals; water plants should send samples to MOE Central Laboratories for analysis.

b) Turbidity - liquid ferric chloride should be sufficiently clear to enable flow-measuring devices to be read without difficulty.

Specifications. Lquid ferric chloride should contain from 32-47% $FeCl_3$ and 11-17% iron.

C) SODIUM ALUMINATE ($Na_2O \cdot Al_2O_3$)

Sodium aluminate is used for coagulation and for corrosion and pH control. There are no AWWA standards for this treatment chemical.

Storage - The solid form may be stored in bags, the liquid in steel or plastic drums.

Precautions - The dust is harmful and protective masks should be used during handling. The chemical tends to cake, and is mildly corrosive when wet.

Testing -

a) Turbidity - solutions of sodium aluminate should be sufficiently clear to enable flow-measuring devices to be read without difficulty.

Specifications. Solid sodium aluminate should contain 55% Al_2O_3, and liquid 32% Al_2O_3.

2. TREATMENT AIDS

Contaminant concentrations in chemicals used as treatment aids are low, and not expected to contribute levels of concern to the treated water, especially in view of the low dosage and, for the most part, intermittent use. Testing of such chemicals upon receipt at water treatment plants is not necessary. However, storage and handling procedures are given below, along with the AWWA specifications and standard number for each chemical.

A) SODIUM CARBONATE (Na_2CO_3) SODA ASH AWWA STANDARD B201-80

Sodium carbonate is used for corrosion control, softening and as a coagulant aid.

Storage - Sodium carbonate in bags or drums, should be stored in a cool, dry area in sealed containers to minimize contact with moisture and air; if moisture is absorbed, the material will become lumpy and difficult to handle and dissolve.

Precautions - Not classified as dangerous but it is dusty and irritates the eyes, nose, lungs and skin; it is mildly corrosive if wet. Goggles and dust respirators are recommended for handling. Soda ash and lime should not be present together, since caustic soda (highly corrosive) may form on combination of the chemicals and moisture.

Specifications. Material should be free of dirt, rocks, twigs or other foreign materials. Manufacturers specifications should provide results of the following tests; the AWWA specifications are given in parenthesis.

Density - light soda ash (0.5 - 0.8 g/mL)
- dense soda ash (0.9 to 1.1 gm/mL)
Sodium carbonate (not less than 99% by weight Na_2CO_3)
Sodium oxide (equivalent of not less than 57.9% Na_2O)
Insoluble material (not to exceed 0.05%)

B) SODIUM BICARBONATE $NaHCO_3$

There are no AWWA Standards for sodium bicarbonate. It is used as an aid in coagulation and for corrosion control.

Storage - Sodium bicarbonate should be protected from moisture in plastic-lined bags and stored in a cool dry place.

Precautions - It should not be allowed to come into contact with aluminum, with which it reacts, otherwise no special handling precautions are necessary.

Specifications. Sodium bicarbonate as $NaHCO_3$ not less than 98.6%.

C) QUICKLIME AND HYDRATED LIME CaO AWWA STANDARD B202-77

Lime is used for pH and corrosion control and for softening.

Storage - Lime should be stored in a cool dry area in sealed containers to minimize contact with moisture and air; it is unstable if left out in the open in unsealed containers.

Precautions - Lime is not classified as a dangerous chemical, but goggles and dust respirators are recommended for handling. Lime and soda ash should not be present together, for caustic soda may form if the chemicals combine in the presence of moisture. The chemical reacts with water when it is mildly corrosive and can burn the skin.

Specifications. Lime is supplied as lumps (density 897-1040 kg/m^3), pebbles (density 960-1024 kg/m^3) or granules (density 800-1120 kg/m^3). Manufacturers specifications should include the results of the following tests; the AWWA specifications are given in parenthesis:

Available calcium oxide (quicklime not less than 80%, hydrated lime not less than 62%)

Insoluble material (not more than 5%)

Slaking test (quicklime shall produce at least a 10°C rise in temperature in 3 minutes, and a maximum temperature within 20 minutes).

Fluoride (such that the fluoride content in distributed water will not be increased more than 0.1 mg/L on lime treatment)

D) ACTIVATED CARBON

Activated carbon is used for taste and odour control organics removal and for dechlorination.

1. POWDERED ACTIVATED CARBON. AWWA STANDARD B600-78

Storage - Powdered activated carbon should be stored in a fireproof building, stacked in rows with aisles between so that each individual bag is accessible for removal in case of fire. No other materials should be in the same building.

Precautions - Strict precautions to avoid contact with strong oxidizing agents (chlorine, hypochlorites, permanganate, ozone and peroxides) are necessary, and mixing with hydrocarbons may cause spontaneous combustion. Dust respirators should be used in handling, and electrical connections in feed rooms should be watertight and dustproof. When moist, activated carbon will absorb oxygen from the air, and appropriate safety measures should be observed when entering enclosed areas containing activated carbon. N.B. A carbon fire may be extinguished using a fine water spray or chemical foam; water jets will spread the smouldering particles.

Specifications. The material should be free of diluents, either soluble or insoluble, and should not impart to the water any contaminants that exceed the limits of the Drinking Water Objectives when combined with levels already in the water. Manufacturers specifications should include the results of the following tests; the AWWA standard is given in parenthesis:

Moisture content (not to exceed 8% by weight at time of shipment)

Apparent density (not less than 0.2 gm/mL nor greater than 0.75 gm/mL)

Absorptive capacity

a) Iodine number (not less than 500)

b) Phenol value (not greater than 3.5)

N.B. Reduction of threshold odour or tannin absorption can be used to measure absorptive capacity. (Reduction of threshold odour by a sample, should be not less than 70% of that of a reference sample, and the tannin value not more than 10% greater).

2. GRANULAR ACTIVATED CARBON AWWA STANDARD B604-74

Storage - Granular activated carbon should be stored in a cool dry area.

Precautions - Strict precautions should be taken to avoid contact with strong oxidizing agents (chlorine, hypochlorites, permanganate, ozone and peroxides, mixing with hydrocarbon may cause spontaneous combustion. When moist, an oxygen-depletion hazard may develop in confined areas.

Specifications. The material shall contain no soluble inorganic or organic substances that would render the treated water unfit for use, and should not impart to the water any contaminants that exceed the limits of the Drinking Water Objectives when combined with levels already in the water.

Manufacturers specifications should include the results of the following tests; the AWWA standard is given in parenthesis:

Moisture content (not to exceed 8% by weight at time of shipment)

Apparent density (not less than 0.36 gm/mL)

Abrasion resistance (retention of average particle size not less than 70% as determined by standard tests)

Absorptive capacity

a) Iodine number (not less than 500)

E) SODIUM HYDROXIDE (NaOH) AWWA STANDARD B501-80

Sodium hydroxide is used for pH and corrosion control.

1. ANHYDROUS SODIUM HYDROXIDE (CAUSTIC SODA)

Storage - Flake, ground, crystal or bead caustic soda should be stored in steel containers, fibreboard drums or polyethylene-lined bags, in a cool, dry area. Protection from moisture is essential, since it will be absorbed, generating heat.

Precautions - Caustic soda in all forms is extremely hazardous and full protective clothing including goggles, face shields, cotton clothing, rubber aprons, boots and gloves are essential. Dilution and dissolution result in heat-generation, and should be carried out with extreme caution to avoid boiling or splattering; emergency eyewash and shower facilities should be provided in the vicinity of handling areas. All personnel should be given detailed instructions on handling procedures.

Specifications. Caustic soda is a white to off-white opaque to translucent solid which rapidly absorbs moisture. Manufacturers specifications should include the results of the following tests; the AWWA standard is given in parenthesis:

Percent total alkalinity as Na_2O (74.4% minimum)
Percent sodium hydroxide as NaOH (96% minimum)
Percent carbonate as Na_2CO_3 (not more than 2%)

2. LIQUID SODIUM HYDROXIDE (CAUSTIC SODA)

Storage - Liquid caustic soda should be stored in glass or steel containers in a cool dry area.

Precautions - Caustic soda in all forms is extremely hazardous and full protective clothing, including goggles, face shields, cotton clothing, rubber aprons, boots and gloves are essential. Dilution results in heat-generation; and should be carried out with extreme caution, to avoid boiling or splattering; emergency eyewash and shower facilities should be provided in the vicinity of handling areas. All personnel should be given detailed instructions on handling procedures. The 50% NaOH begins to crystallize at 12^{O}C, which can cause problems in Canadian winters.

Specifications. Caustic soda is available in liquid form in concentrations from 12% to 50% NaOH. Manufacturers specifications should include the results of the following tests; the AWWA standard is given in parenthesis:

Percent total NaOH (at least 50%)

N.B. In Canada, percent total NaOH should be as specified in the order.

F) FLUORIDATION CHEMICALS

Fluoridation chemicals are added for prophylactic purposes to prevent dental caries in consumers.

1. HYDROFLUOROSILICIC ACID (H_2SiF_6) AWWA STANDARD B703-71

Storage - Hydrofluorosilicic acid (fluosilicic acid) should be stored in a well-ventilated room in containers of structural carbon, Hastolley C, Durimet 20 or such rubbers and plastics as recommended by the manufacturers.

Precautions - Since hydrofluorosilicic acid is very corrosive and harmful to the skin and eyes, protective clothing and eyewear are essential. Feed equipment should be the same as that specified for storage containers. Emergency showers should be available in the vicinity of handling areas.

Specifications. Hydrofluorosilicic acid should be clean and free of visible suspended matter, and generally contains from 20 to 30% H_2SiF_6. Manufacturers specifications should include the results of the following tests; the AWWA specifications are given in parenthesis:

Heavy metals (mercury, lead, bismuth and copper expressed as lead) (not more than 0.020%)

Percent H_2SiF_6 by weight (between 20 and 30%).

2. SODIUM SILICOFLUORIDE (Na_2SiF_6) AWWA STANDARD B702-74

Storage - Fibre drums, paper bags or barrels may be used to store sodium silicofluoride in a cool dry place; it is hygroscopic and tends to lump, rendering use difficult.

Precautions - Sodium silicofluoride is poisonous if inhaled or swallowed and containers should be labelled appropriately. Dust respirators and goggles should be worn during handling and personnel should wash thoroughly after handling the material. Spills should be cleaned up immediately. Medical attention is required immediately if the solid chemical is ingested.

Specifications. Sodium silicofluoride is a white powder, and should contain no stones, sticks, paper or other foreign matter. It may contain an anti-caking agent if this will not interfere with its application to drinking water, and it is non-toxic and odourless. Manufacturers specifications should include the results of the following tests; the AWWA specifications are given in parenthesis:

Density (approximately 75 lb/cu. ft.)

Standard sieve #40 (420 μm) (at least 98% passing through)

Standard sieve #325 (44 μm) (less than 25% passing through)

Sodium silicofluoride (Na_2SiF_8) (minimum 98% corresponding to approximately 59.4% fluoride ions)

Insoluble matter (not to exceed 0.5%)

Moisture (not to exceed 0.5%)

Heavy metals (mercury, lead, bismuth and copper expressed as lead) (not more than 0.05%).

NOTE. Much of the material used to produce this Appendix was obtained from the AWWA Standards for the individual chemicals (American Water Works Association, 6666, West Quincy Avenue, Denver, Colorado, 80235).

These standards include such information as:

-Definition of the Standard and an outline of the basis for the purchase, and/or rejection of the chemical

-Description of the material, including impurities and size

-Sampling, packing, shipping and marking requirements for various chemicals

-Testing methods to determine the quality of chemicals

A Wall Chart, listing common water treatment chemicals, chemical names and formulae, containers precautions uses etc. in abbreviated form is available from "Water and Pollution Control", 1450, Don Mills Road, Don Mills, Ontario, M3B 2X7 at $5.00 each (bulk rates available).

Experimental and Environmental Modelling Studies
of
Hazardous Substances in Ontario

D. Mackay
S. Paterson
B. Cheung
W. Y. Shiu
Department of Chemical Engineering and Applied Chemistry
University of Toronto

ABSTRACT

It is becoming widely accepted that assessment of the behaviour, and hence the effects, of toxic chemicals requires the use of mathematical models which may be applied to actual or evaluative environments. This is necessitated by the large number of potential substances of concern and the wide variation in their physical-chemical and toxicological properties. In this project a version of the evaluative Fugacity Model has been developed and is being applied to a number of selected chemicals, namely the chlorobenzenes, the chlorophenols, mirex, atrazine and trichloroethylene. The evaluative model is specifically designed to be applicable to the Ontario environment. Attempts are being made to obtain emission rate data and environmental monitoring data which can be used to calibrate the model.

A novel aspect is quantification of concentration heterogeneities and the inclusion of such concentrations as lognormal or Weibull distribution functions. The feasibility of presenting the data in computer - generated colour contour form is being investigated.

Finally, methods of including toxicity information are being examined and will be discussed.

INTRODUCTION.

In late 1982 we initiated a project with MOE support to develop a computer modelling system which could be used to assess the behaviour, and ultimately effects, of toxic contaminants in Ontario. This is essentially a progress report and a description of current and future work.

It is now clear that environmental "management" of toxic substances presents a severe scientific and organizational problems to Government Agencies. It is an issue quite unlike any previous issue which has been successfully addressed. Some features which cause difficulties are:-

1. There is a large number of recognized and potential toxicants, most lists running to several hundred members.
2. In many cases the "member" is a homologous series, for example the 209 polychlorinated biphenyls, or the chlorinated dibenzofurons, dibenzodioxins or toxaphenes.
3. Rarely is there adequate information on the partitioning, reaction and inter-media transport properties of these substances.
4. Rarely is there adequate toxicological information, nor is there an appreciation of the dominant routes of exposure or dosage.
5. Many of the contaminants migrate readily between air, water, soil, sediment and biota thus they do not fit neatly into organizational structures designed to assess "water or air pollutants" such as phosphorous or NO_x.
6. The environment is a very complex assembly of media of

varying composition and condition (e.g. temperature).

7. Sources are usually of unknown magnitude may be "point" or "non-point" or both in nature and may be intermittent in nature. Certain locations may be particularly contaminated.
8. Monitoring data are sparse, expensive to obtain and may stretch "state of the art" analytical methods.

It is clear that the resources which can be devoted to any one toxicant must be relatively modest and can not approach those which have been devoted to toxicants such as mercury, phosphorus, or sulfur oxides.

We have become convinced that the only realistic approach is to develop a number of mathematical models or partial representations of reality in which the general behaviour of each toxicant can be computed from known input parameters. The art of such modelling is to devise models in which the correct key processes are described and less important processes are ignored or appropriate simplifying assumptions made. The critical decision is "what to leave in" vs "what to leave out". This requires, judgement based on experience.

In a series of recent publications (Mackay and co-workers 1978-83) we have advocated the use of the fugacity concept for simplifying environmental modelling. Accordingly, in this project we are extending these models and applying them to Southern Ontario conditions for selected substances.

The general aim is to gather information on the following aspects for a defined study area and synthesise them into a model which can be subjected to some form of validation

(i) Source locations, amounts, and emission times, including advective inflow in air and water (especially the Niagara River).

(ii) Relevant physical chemical pro½perties.

(iii) Toxicological properties.

(iv) Monitoring data in the study area.

We discuss below each aspect in turn.

Study Area/Evaluative Model

Two models have been assembled. First is a purely evaluative model comprising the various environmental compartments described by Neely & Mackay (1982) as shown in Figure 1. This is the closest available to a standardised evaluative environment. Second is a model representing part of Southern Ontario as shown in Figure 2. The boundaries are somewhat arbitrary but it is believed that they include most of the regions of substantial emission and concern. The French River forms the northern boundary. All of Lake Ontario and Lake St. Clair are included as well as Lake Erie north of the international boundary, Georgian Bay and part of Lake Huron. This region may be redefined later if it is considered necessary. Lake Ontario is the only one of the Great Lake included along with Lake Simcoe and the other inland lakes. In total the area is approximately 13 x 10^{10} m^2 of land and 5.0 x 10^{10} m^2 of water containing these estimated volumes (assuming reasonable depths, heights and volume fractions).

air	3.7 x 10^{14} m^3
water	5.0 x 10^{12} m^3
soil	2.0 x 10^{10} m^3
sediment	1.5 x 10^{9} m^3
suspended sediment	2.5 x 10^{7} m^3
aquatic biota	5.0 x 10^{6} m^3

The Benchmark Chemicals

Following discussions with the Ministry we have selected the following chemicals for study. In each case the chemical(s) have a dominant environmental fate property as indicated

mirex	sediment contaminant
atrazine	herbicide, water and soil contaminant
trichloroethylene	air contaminant
chlorobenzenes	series of contaminants of varying hydrophobicity tending to accumulate in air or sediments and aquatic biota.
chlorophenols	series of water contaminants of varying hydrophobicity of unusual toxicity, precursors to dioxins

It is possible that other substances may be added at a later stage if appropriate.

Physical-Chemical Properties

An extensive literature search has been conducted for these compounds and all available physical-chemical property data assembled. In some cases it has been necessary to measure the properties.

The reports obtained from this literature review have been standarized to take the following format.

A general narrative is written describing the properties and other features of the chemical, including review (with references) of other studies, and matters of historical and other significance. At various stages the narrative is punctuated by "data sheets" which give the following information in a format which may be directly incorporated into the fugacity models.

(1) Sources (emissions categorized into air, water and soil in mol/h for the Province and the evaluative environments).

(2) Fugacity Capacities (Z values) (partitioning information between phases).

(3) Reactivity (k values) (first order degradation rate constant for each relevant reaction in each phase).

(4) Advection Information on "background" concentrations and flows in air and water.

(5) Interphase transport data (D values), Mass transfer coefficients and/or uptake times.

(6) Hazard/Toxicity Information on concentrations and durations or uptake rates in air, water and food which have been assessed as having defined (or no) adverse effects.

(7) Monitoring Data Information on prevailing concentrations in the Province in various compartments in histogram form of frequency versus concentration.

Reports have been written in draft form for all substances.

When compiling data on the properties of the substances, we have found that there are considerable variations in the values reported, especially for solubility, vapour pressure, octanol-water, sorption and bioconcentration partition coefficients. We have devised a system for compiling the data, weighting it according to perceived reliability then selecting the best set of mutually consistent values. This has proved to be an interesting aspect and will, we believe, be of value to the Ministry (and others) in critically assessing physical chemical property data which may be received or measured for compounds of environmental interest.

Essentially, the method is an application of the maximum likelihood principle to the data. We have sought assistance from Professor Alan Wilks of the Department of Statistics at Princeton University in this regard.

Fugacity Models

A modified level III fugacity model is being compiled which is appropriate for application to Southern Ontario. The compartments included are: - soil, water (including Lake Ontario), air (to a height of 2 km), sediments, suspended sediment, atmospheric particulates, aquatic biota and terrestrial biota. The simplest level I and II models are also being compiled. Degradation reactions in all phases can be included. Interphase transport reactions included are the exchanges between: - air and water (by diffusion and rainfall), air and soil (by diffusion and rainfall), air and atmospheric particles, air and terrestrial biota, soil and water (runoff), soil and terrestrial biota, water and suspended sediment, water and aquatic biota and sediment and water, suspended sediment and (bottom) sediment atmospheric particles and water, atmospheric particles and soil. Provision is made for the introduction of chemical into air, water and soil compartments. Background or "out of Province" inputs may also be included.

This model is being programmed in BASIC on our IBM Personal Computer and should be readily transferable to other systems.

It is also planned to develop a Level IV model which can be used to obtain data on the time-response characteristics of each phase.

Treatment of phase heterogeneities and incorporation of monitoring data.

A difficulty which arises from the fugacity model is that the phase concentrations obtained are homogeneous, i.e. a constant value applies at all locations. In practice, of course, there are wide variations in concentrations depending on the proximity to sources. This is reflected in the monitoring data which show this range of concentration.

Our approach to treating this "problem" is to compute separately a distribution of concentrations in each principal compartment of air, soil, sediment and water. The other compartments, while important as transfer media (e.g. atmospheric particles) or for socioeconomic reasons (e.g. fish) are not significant "sinks" for chemicals in a mass sense. Their concentrations generally reflect the concentrations in the dominant surrounding compartment (e.g. fish in water). We have investigated the use of normal, log-normal and Weibull distribution functions for characterizing concentration variations within a phase. A constraint must be satisfied that the total amount in a compartment is that indicated by the model (i.e. the same mean concentration applies) but the "variance" (or equivalent) can be selected as appropriate in each case.

We have found that the Weibull distribution is particularly convenient because it exists in analytical form in both differential and integral versions. An analysis has been undertaken of mirex concentration data in the sediments of Lake Ontario. A further stage is the assignment of the concentration distributions to specific locations within the Lake using knowledge of source locations and current directions. This enables a concentration contour diagram to be devised (using available computer graphic techniques) and compared with the actual data obtained by Thomas et al (1983). Figures 3 and 4 illustrate this approach. We hope to extend it to other phases such as air and water.

Hazard Assessment

This is the most distant goal and at present we have only a rather indistrinct impression of the system which may be advised. It is appropriate, however, at this time to expose present ideas for criticism and comment.

From the fugacity models come assessments of the likely prevailing chemical concentrations in air, water, fish, etc., which arise from specified emissions. This could take the form of statements of the form: - The average concentration of compound x will be y mol /m^3 in water but with a distribution of concentrations as shown in Figure 5. Figure 6 illustrates the distribution of mirex concentration in the sediment of Lake Ontario fitted to a Weibull curve. ([illegible] as 1983).

From a review of the compound's toxicology comes an adverse effect distribution of concentrations ranging from an acute toxicity at the upper end to a "no effect level" or (lower) guideline or regulation for "acceptable" water concentrations at the lower end. Juxtaposing these two concentration distributions as is shown in Figure 7 reveals the proximity of the prevailing (or potential) Provincial concentration distribution to the range of concentrations of toxicoloical concern. If the two ranges are close (or overlap) it is apparent that some parts of the Province are (or may be) exposed to undesirably high exposures through the water environment. As the ranges more apart the solution becomes less serious. The seriousness of the situation is thus expressed as the proximity of these concentration distributions. No attempt is made to predict effects e.g. incidence of disease, since this is probably impossible. It should be possible to establish relative priorities e.g. if the distributions for benzene are closer than for naphthalene, benzene is probably a more problematic chemical and may deserve more attention.

This analysis can be applied separately to the air, water and food (i.e. terrestrial and aquatic biota) compartments. An assessment can be made of uptake or exposure to each medium (e.g. a) g/yr by air breathing, b) g/yr by water drinking, c) g/yr by fish eating, d) g/yr by meat eating, etc. The dominant exposure medium then becomes apparent. It is suspected, for example, that this may be air for trichloroethylene, water for chlorophenol, and fish for hexachlorobenzene. More attention can then be focussed on that medium and on areas of the Province where that medium is found to be, or suspected to be most contaminated. It may then be appropriate to devote more monitoring effort to these regions and compartments.

In most cases the conclusions will be self-evident and it is suspected that existing monitoring programs already take these factors into account on a "common sense" basis. For some chemicals data gaps may be revealed and for new chemicals a prior assessment can be made of likely dominant routes of human exposure. Situations which have been accidentally overlooked may be identified.

It is emphasised that at this point we are far from having achieved this goal but we believe that it is useful at this stage to expose these ambitions for critical comment.

References

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4. Mackay, D., Joy, M. and Paterson, S., "A Quantitative Water, Air, Sediment Interaction (QWASI) Fugacity Model for Describing The Fate of Chemicals in Lakes", Chemosphere 1983, 12, 981.
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6. Mackay, D., Paterson, S. and Joy, M., "Application of Fugacity Models to the Estimation of Chemical Distribution and Persistence in the Environment", in Fate of Chemicals in the Environment, eds. Swann, E.L. and Eschenroeder, A., ACS Symposium Series 225, 1983, 175.
7. Thomas, R., Canada Centre for Inland Waters, Burlington, Canada, 1983 Personal Communication.

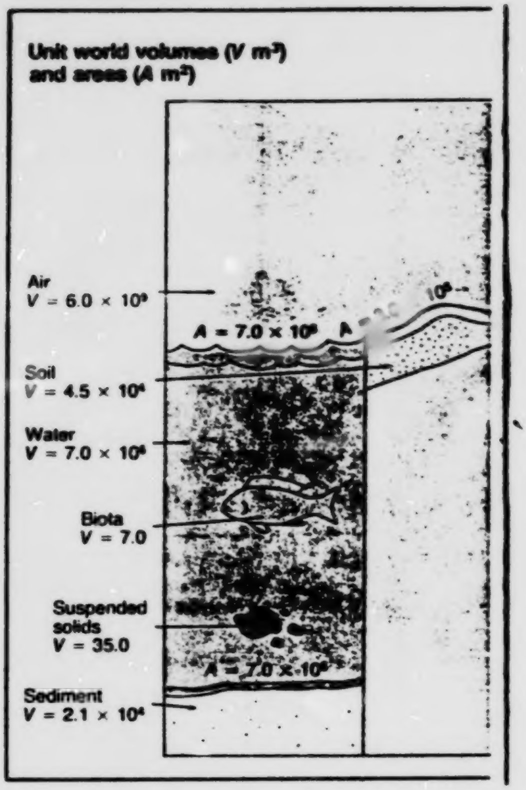

Unit world volumes (V m^3)
and areas (A m^2)
Air
$V = 6.0 \times 10^9$
$A = 7.0 \times 10^6$
A
10^6
Soil
$V = 4.5 \times 10^4$
Water
$V = 7.0 \times 10^6$
Biota
$V = 7.0$
Suspended
solids
$V = 35.0$
$A = 7.0 \times 10^6$
Sediment
$V = 2.1 \times 10^4$

Figure 1. Evaluative Model or Unit World

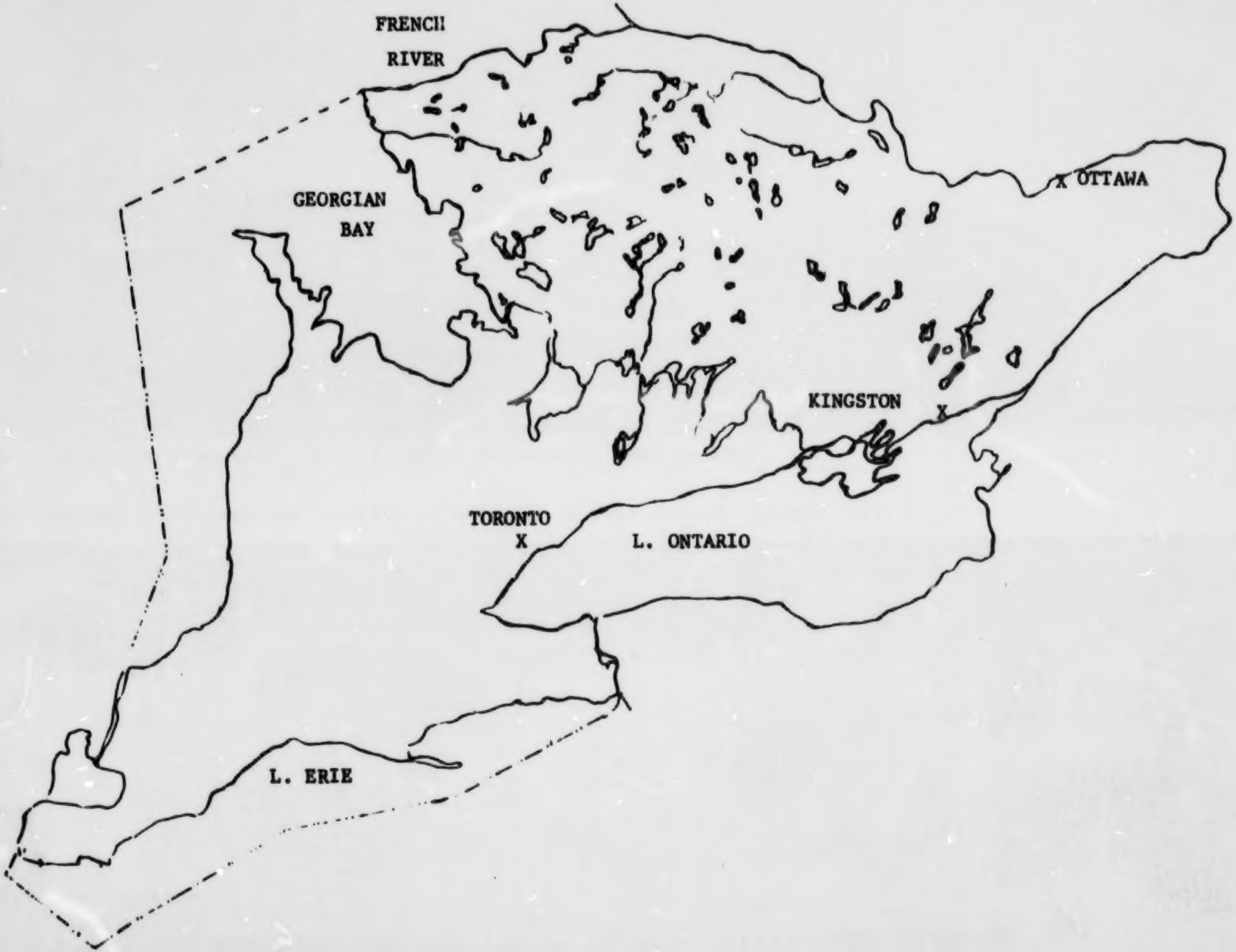

FRENCH
RIVER
GEORGIAN
BAY
X OTTAWA
KINGSTON
X
TORONTO
X
L. ONTARIO
L. ERIE

Figure 2. Map of Southern Ontario Study Area

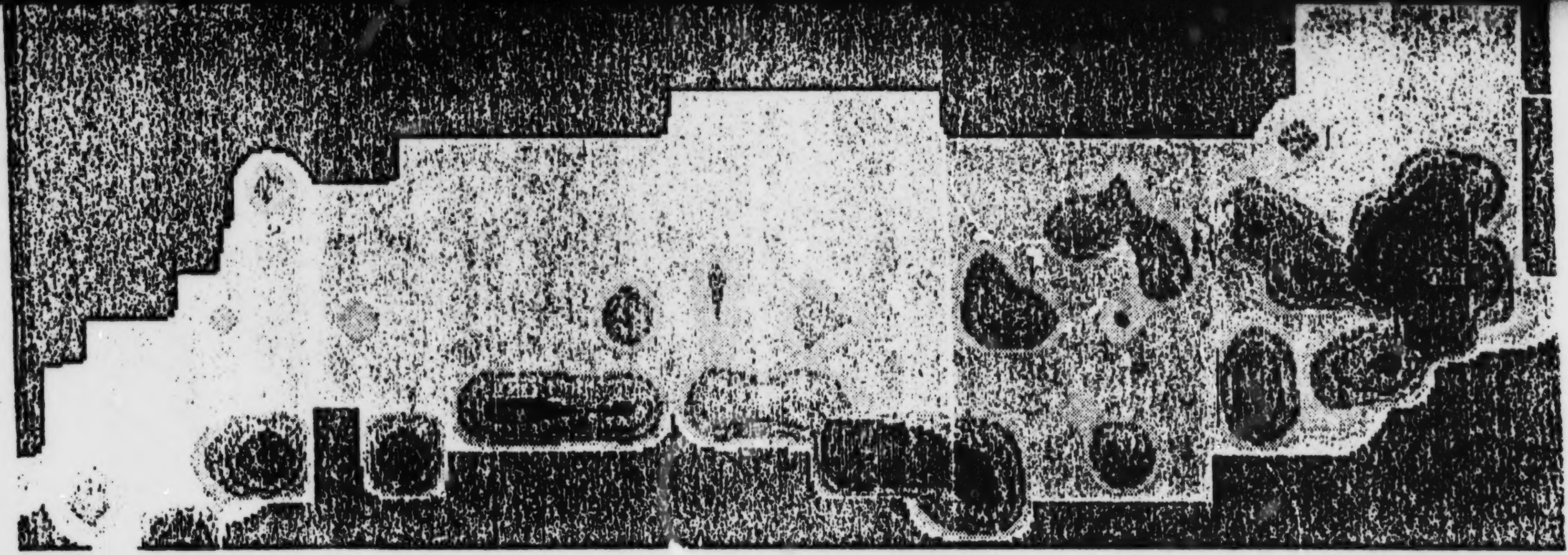
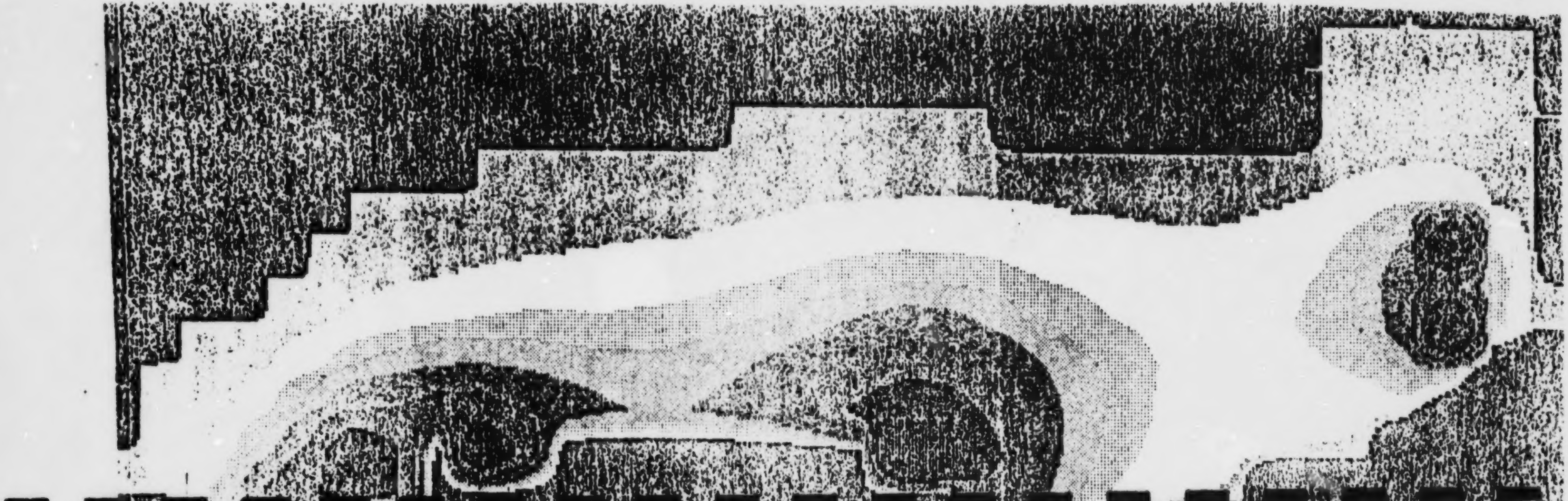


Figure 3. Actual Concentrations of mirex in sediment of Lake Ontario (Thomas 1983)

Figure 4. Predicted concentration of mirex in sediment of Lake Ontario



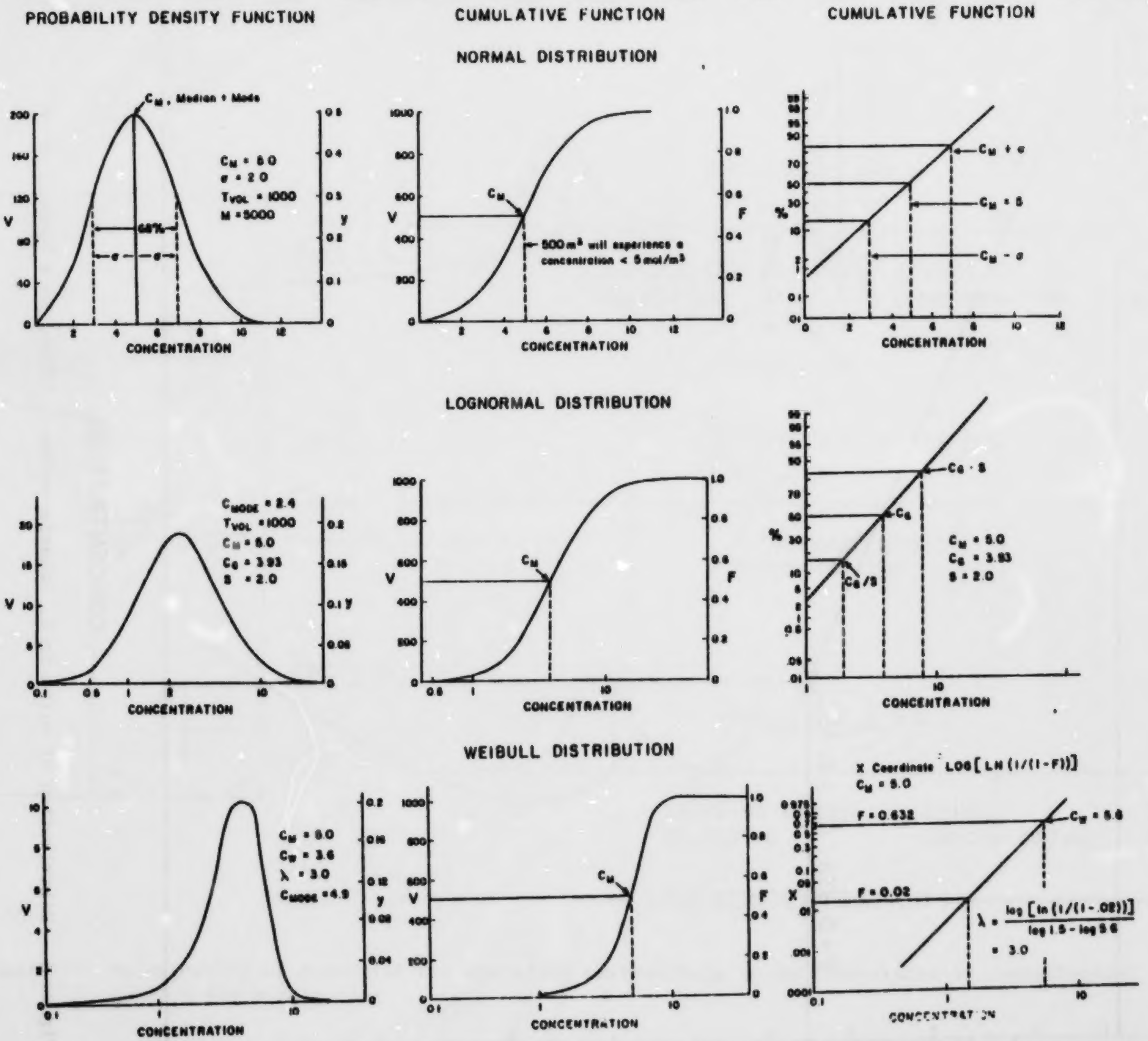


Figure 5. Distribution Functions for characterising concentration variations within a phase

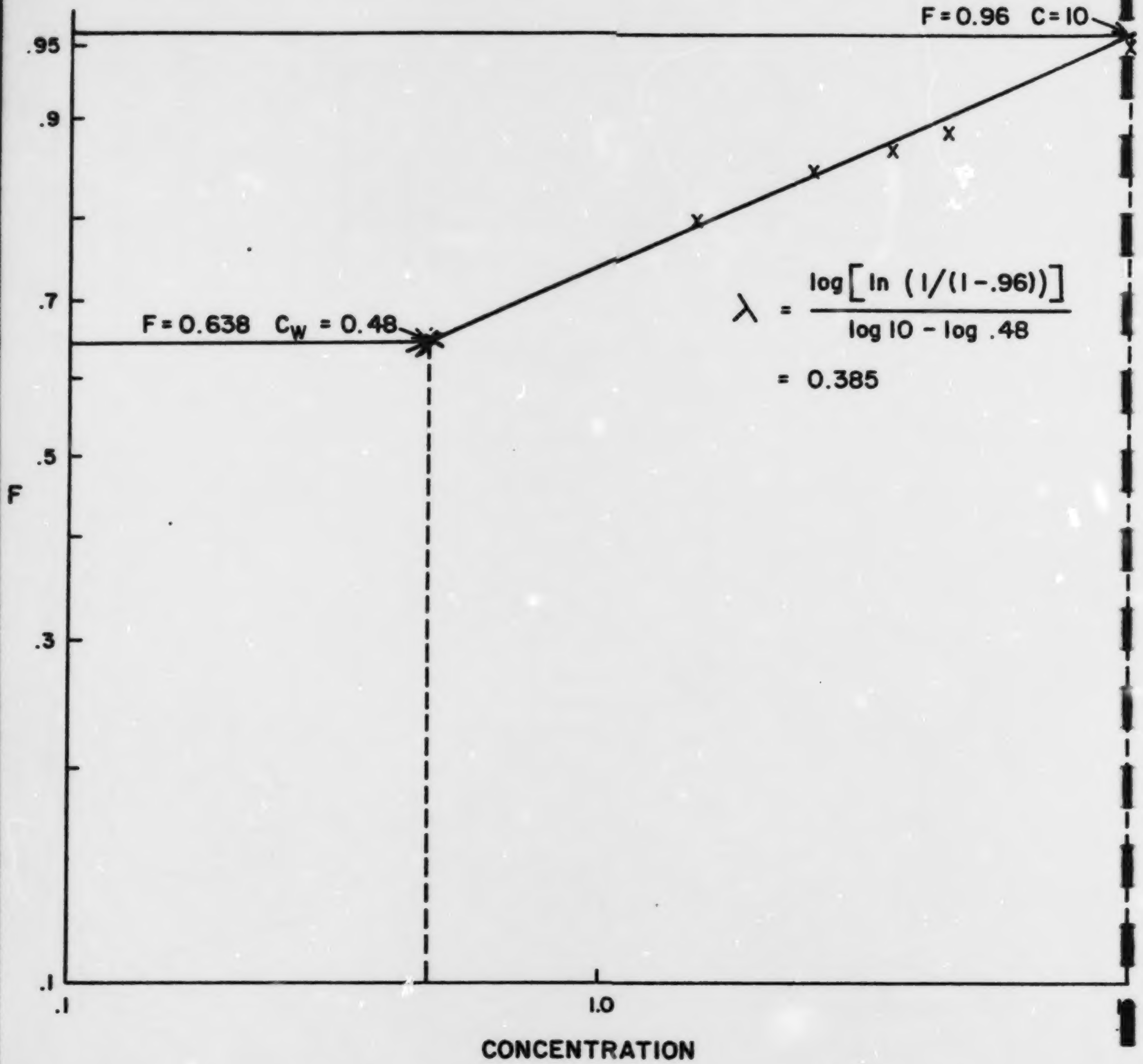


Figure 6. Distribution of mirex in L. Ontario sediment fitted to a Weibull curve

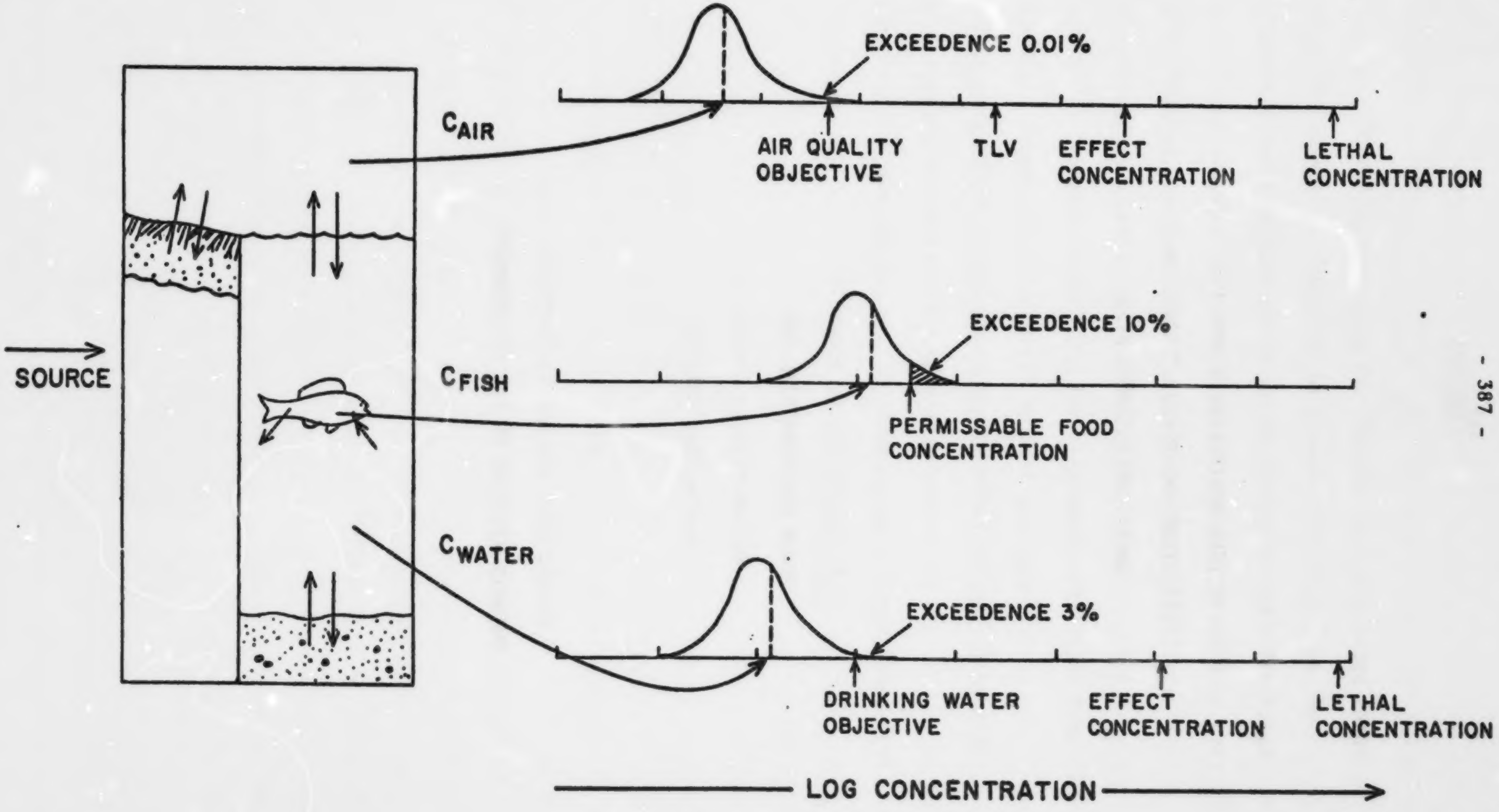

SOURCE
C_{AIR}
EXCEEDENCE 0.01%
AIR QUALITY OBJECTIVE
TLV
EFFECT CONCENTRATION
LETHAL CONCENTRATION
C_{FISH}
EXCEEDENCE 10%
PERMISSABLE FOOD CONCENTRATION
C_{WATER}
EXCEEDENCE 3%
DRINKING WATER OBJECTIVE
EFFECT CONCENTRATION
LETHAL CONCENTRATION
LOG CONCENTRATION

Figure 7. Relationship of potential concentration distribution to concentrations of toxicological concern for a pollutant

REVISED MONITORING SCHEME FOR PERSISTENT AND TOXIC ORGANICS IN GREAT LAKES SPORTS FISH

by

J.A. Coburn and H. Huneault
ZENON ENVIRONMENTAL INC.
845 Harrington Court
Burlington, Ontario

and

Gerald Rees and George Crawford
ONTARIO MINISTRY OF THE ENVIRONMENT

ABSTRACT

Screening protocols for the analysis of a broad range of synthetic organic compounds have been identified from the literature and have been evaluated on fortified and unfortified fish tissue samples. Recoveries of the trace organics have been determined at low parts per million and parts per billion concentrations. Sample extracts were processed using gel permeation chromatography (GPC) for elimination of lipids from the trace organic fraction prior to full scan gas chromatographic-mass spectrometric (GC/MS) analysis. Data will be presented on the sample preparation protocols, GPC fractionation and preliminary GC/MS analyses of Great Lakes fish samples.

Over the past two decades the number of detections of trace organic contaminants in fish samples has been increasing considerably. These analysis have detected the presence of PCB, DDT and metabolites, chlordane, benzene hexachloride isomers including lindane, dieldin, endrin, chlorinated benzenes, mirex, mirex metabolites, and chlorinated styrenes (COA Report, 1981). The International Joint Commission has reported that in addition to the usual chlorinated hydrocarbons, phthalic acid esters, volatiles such as chloroform, bromoform and tetrachloroethylene, Toxaphene, polyaromatic hydrocarbons (PAH) and alkylated and chlorinated PAH and a broad array of aliphatic hydrocarbons have been identified in fish.

Numerous authors have more recently reported the presence of 2,3,7,8-tetrachlordibenzo-p-dioxin at part pere trillion concentrations (Kuehl, 1981; Ryan, 1982; Harless, 1980). Additional analysis have also revealed the presence of ultra-trace (part per trillion) levels of other chlorinated dioxins and polychlorinated-dibenzofurans (Kuehl, 1981).

Kuehl,(1980) has also reported the presence of hexachloro and heptachloro styrenes, pentachlorophenol and pentachlorobenzyl alcohol in fish samples in the Great Lakes watershed.

Chlorinated toluenes and chlorofluorotoluenes have been reported in fish tissue samples collected from the Niagara River (Yurawecz, 1979). Polychlorinated diphenyl ethers have been reported in sediments and fish collected from Whitby Harbour in Lake Ontario (Coburn 1981) with levels higher than the PCB.

Polychlorinated naphthalenes have been detected and identified in fish from the Titabawassee River which flows into Saginaw Bay of Lake Huron (Kuehl, 1980) at very low parts per billion concentrations.

Hexachlorobutadiene and hexachlorocyclopentadiene have been reported to be in numerous hazardous waste landfills along the Niagara River in the New York State (Intereagency Task Force on Hazardous Wastes, 1979) however only the hexachlorobutadiene was detected in the fish samples from the Niagara River (Yurawecz, 1979).

A comprehensive analysis of water and sediments adjacent to hazardous waste landfills in Niagara Falls, N.Y. (Elder, 1981) has detected chlorobenzenes, chlorotoluenes, polyaromatic hydrocarbons, PCB, chlorophenols, fluorinated aromatics, and a series of unchlorinated and chlorinated benzyl derivatives. There have been no reports in the literature of the detection of this latter group of organics in fish samples from Niagara River or Lake Ontario.

The report of Hesselberg, (1982) also identified two halogenated contaminants found in Great Lakes fish samples which were not previously identified as pollutants of concern by the International Joint Commission. They were 3-chloro-1-propynyl-cyclohexane and 3-bromomethyl-cyclohexene with the latter compound being detected in all Great Lakes samples tested.

These reports reveal that as analytical techniques continue to improve, there are increasing detections of trace organic chemicals in the fish of the Great Lakes at ever decreasing concentrations.

There have been basically four main techniques employed in the extraction of persistent and toxic organics such as DDT, chlordane, PCB, mirex, etc. from biological tissues. These are:

i) mechanical homogenization of the tissue with a solvent or mixture of solvents,

ii) cold HCI acid dissolution of the biological tissue followed by a partitioning into an organic solvent.

iii) solvent elution from a column of biological tissue mixed with anhydrous sodium sulphate,

iv) steam distillation of biological tissue (in an aqueous solution) followed by condensation of the water vapour and trace organics and flow through partitioning of the organics into a non-water soluble solvent.

The first three techniques, because of the presence of high levels of lipids and fats, require the separation of these coextractives from the extract prior to separation or fractionation of contaminants and anlysis. The two most commonly employed techniques for lipid - contaminants separation are:

i) acetonitrile - petroleum ether partitioning usually followed by Forisil column chromatography (This technique is referred to as the Mills, Onley, Gaither technique).

ii) automated gel permeation chromatography on Bio-Beads SX-3 eluting with dichloromethane or dichloromethane; cyclohexane solvent mixtures.

EXPERIMENTAL SECTION

Fortified "clean" fish and unfortified "real" fish samples were analyzed using three analytical techniques. Model compounds, representing five chemical classes were used for fortification at concentration levels of roughly 100 ng/g and 20 ug/g. The "clean" sample was a lake trout from Lake Opeongo in Algonquin Park and the "real" sample was a lake trout from the north shore of Lake Ontario. Table 1 shows the model compounds and surrogate standards used in this study while Table 2 shows the concentrations of these compounds at the two fortification levels.

For the analysis of the high level spiked samples 5 grams of tissue were extracted and the extract taken to a final volume of 1.0 mL. For the low level spike and the unspiked "real" fish 5-10 grams of tissue were extracted and the final extract volume adjusted to 200 uL.

The three techniques evaluated are briefly presented below:

Acid Digestion

Fish tissues were digested overnight in cold HCl and extracted with 25% dichloromethane in hexane (v/v). Entrained acid was neutralized with sodium bicarbonate and the extract reduced in volume by rotary evaporation. Volumes were adjusted

TABLE 1

MODEL COMPOUNDS AND SURROGATE STANDARDS

Class	Contaminant	Low Level Spike	High Level Spike	"Real" Sample
Volatiles	Trichlorobenzene	X	X	-
	Hexachlorobutadiene	X	X	-
	Hexachlorobenzene	X	X	-
Chlorophenols	2-Chlorophenol 3,4,5,6-D4*	-	-	X
	2,4 Dichorophenol	X	X	-
	2,4,5 Trichlorophenol	X	X	-
	Pentachlorophenol	X	X	-
Aromatic Amines	Diphenyl Amine	X	X	-
Organo chlorines & PCB	Mirex	X	X	-
	p,p'-DDE	X	X	-
	Dieldrin	X	X	-
	Aroclor 1242,54,60	X	X	-
Polyaromatic Hydrocarbons	Fluoranthene	X	X	-
	Phenanthrene D-10	X	X	-
	Anthracene D-10*	-	-	X

* Internal Standards

TABLE 2

SPIKING LEVELS OF FORTIFIED COMPOUNDS

FOR LOW AND HIGH LEVEL SPIKING OF "CLEAN" FISH HOMOGENATE

MODEL COMPOUNDS	Low Level Spikes ng/g	High Level Spikes ug/g
Dichlorophenol	147	26
Trichlorophenol	102	19
Pentachlorophenol	78	14
Diphenyl Amine	119	21
Trichlorobenzene	89	16
Hexachlorobutadiene	177	31
Hexachlorobenzene	110	20
Mirex	98	17
p,p-DDE	98	17
Dieldrin	98	17
Fluoranthene	124	22
Phenanthrene d10	39	7

to 10 mls 1:1 dichloromethane/cyclohexane. A 1 mL. portion was removed for lipid determination and a seven mL. aliquot was taken for GPC. The GPC eluate collected was rotary evaporated to approximately 2 mL., transferred to a reacti-vial with rinsings, with final volumes achieved by blowing down with a gentle stream of nitrogen.

Polytron Homogenization

Contaminants were extracted from the tissue using dichloromethane, sodium sulphate and a polytron tissue homogenizer. The dichloromethane extract was reduced in volume by rotary evaporation and made up to 10 mL. with 1:1 cyclohexane/dichloromethane in a calibrated centrifuge tube. A 1 mL. portion of the centrifuged extract was taken for lipid analysis with a 7 mL. aliquot of the remainder of the extract processed through gel permeation. Once again, the GPC eluate was rotary evaporated to 2 mL and quantitatively transferred to a reacti-vial where nitrogen gas was used to gently evaporate to final extract volume.

Steam Distillation

Fish homogenates were weighed and transfered to 50 mL round bottom flasks with rinses of organic-free water. The volume of water was adjusted to 300 mL. Three mL organic-free water and 10 mL hexane were charged into the condenser portion of the apparatus. Steam distillation of the samples continued for 3 hr from the time distillation began. At the end of this time heating was stopped and the system allowed to cool. The water and hexane was drained from the condenser into a centrifuge tube.

The organic layer was removed and reduced to the desired final volumn. The steam distillation extracts weree not processed through the GPC prior to GC/MS analysis.

The lipid removal step used throughout this study was by automated gel permeation chromatography (GPC) on Bio-beads SX-3 eluting with 1:1 dichloromethane:cyclohexane. Recovery studies were pereformed on the GPC at the same levels tested on the fish.

The GC/MS analyses were performed on a Finnigan 4510 using a 30 M SE-54 capillary column directly interfaced to the ion source. Electron impact (70 eV) spectra were obtained over the mass range of 90-550 A.M.U. scanning once per second. The column temperature program was 70°C for 2 min., 70°C to 280°C at 10°C per min. with a hold time of 10 min..

RESULTS AND DISCUSSION

Fortification Studies

GC/MS analysis of samples processed using the polytron homogenization procedure produced good mass spectra for all compounds for both the low level and high level spikes. The acid dissolution performed equally well for all compounds except diphenyl amine which would not be extractable from the acid digestion solution. Steam distillation failed to recover the higher chlorinated phenols, and resulted in very poor recoveries of diphenyl amine mirex, p,p'-DDE, dieldrin and fluoranthene. The recovery data for the three methods are reported in Table 3.

TABLE 3

RECOVERIES OF FORTIFIED COMPOUNDS

FOR LOW AND HIGH LEVEL SPIKING OF "CLEAN" FISH HOMOGENATE

MODEL COMPOUNDS	LOW LEVEL			HIGH LEVEL		
	Acid Digestion Recovery %	Polytron Homogenization Recovery %	Steam Distillation Recovery %	Acid Digestion Recovery %	Polytron Homogenization Recovery %	Steam Distillation Recovery %
Dichlorophenol	79.0	66.1	21.0	87.2	98.1	24.1
Trichlorophenol	78.3	63.0	-	78.9	64.3	-
Pentachlorophenol	105	100	-	81.7	40.0	-
Diphenyl Amine	-	36.5	47.0	-	83.8	14.0
Trichlorobenzene	90.7	74.4	49.0	76.6	83.8	61.4
Hexachlorobutadiene	64.7	68.6	40.0	70.1	83.3	62.6
Hexachlorobenzene	42.4	39.0	55.2	43.0	68.5	42.3
Mirex	36.8	44.7	4.5	83.1	94	3.5
p,p-DDE	*	*	*	94.7	93.0	9.9
Dieldrin	22.2	44.4	23.4	79.6	86.8	12.3
Fluoranthene	46.2	43.8	19.3	93.9	92.6	6.3
Phenanthrene d10	47.8	43.3	60.0	88.4	81.4	40.3

* High level in fish homogenate blank

Polychlorinated biphenyls (PCB) were also tested for recovery by the three methods. This data, reported in Table 4, shows that while both the acid digestion and polytron homogenization resulted in good recoveries for the dichloro- to heptachlorobiphenyl, isomers the steam distillation recoveries decreased significantly with increasing chlorination and the overall recovery of the PCB was less than 50%.

The results for the duplicate GPC spike recovery test are reported in Table 5. In all cases, the recoveries from the GPC were lower than the recoveries found in the overall fish fortification, extraction and GPC studies. This has been observed previously and seems to be related to the presence of lipid materials acting as a "keeper" in all extract concentration steps.

Figure 1 and 2 show the reconstructed ion chromatograms of the high level spike and low level spike respectively. From the RIC plots, the peaks of the model compounds are easily observed for the high level spike while the compounds are far less apparent in the low level spike samples. Foreground-background subtraction routines assist in further defining these lower concentration compounds and in providing useful mass spectra.

Naturally Contaminated Fish Study

The real fish homogenates demonstrated little variability in terms of contaminants extracted by polytron homogenization or acid digestion. As in the fortification study, the steam distillation extraction procedure was far less efficient in

TABLE 4

PCB RECOVERY

PCB Isomer	# of Isomers	Acid Digestion % Rec	Polytron Homogenization % Rec	Steam Distillation % Rec
Cl_2	3	74.3	65.5	91.8
Cl_3	4	85.4	70.5	73.1
Cl_4	4	93.5	85.7	35.9
Cl_5	5	94.0	92.7	19.3
Cl_6	6	102	103	4.7
Cl_7	4	103	103	-
Mean		92.0	86.7	37.5

Total PCB spike of 60 ug

TABLE 5

GPC SPIKE RECOVERIES FOR MODEL COMPOUNDS

MODEL COMPOUND	GPC Spike #1 %	GPC Spike #2 %	Average %
Dichlorophenol	78.5	67.0	72.8
Trichlorophenol	60.0	43.0	51.5
Pentachlorophenol	60.7	51.5	56.1
Diphenyl Amine	86.0	74.8	80.4
Trichlorobenzene	76.0	68.6	72.4
Hexachlorobutadiene	68.8	76.6	72.4
Hexachlorobenzene	74.5	70.3	72.4
Mirex	74.3	69.5	71.9
p,p'-DDE	95.0	72.5	83.4
Dieldrin	72.7	79.3	76.0
Fluoranthene	93.0	84.8	88.9
Phenanthrene d10	82.8	71.3	77.1

FIGURE - 1 RIC HIGH LEVEL SPIKE

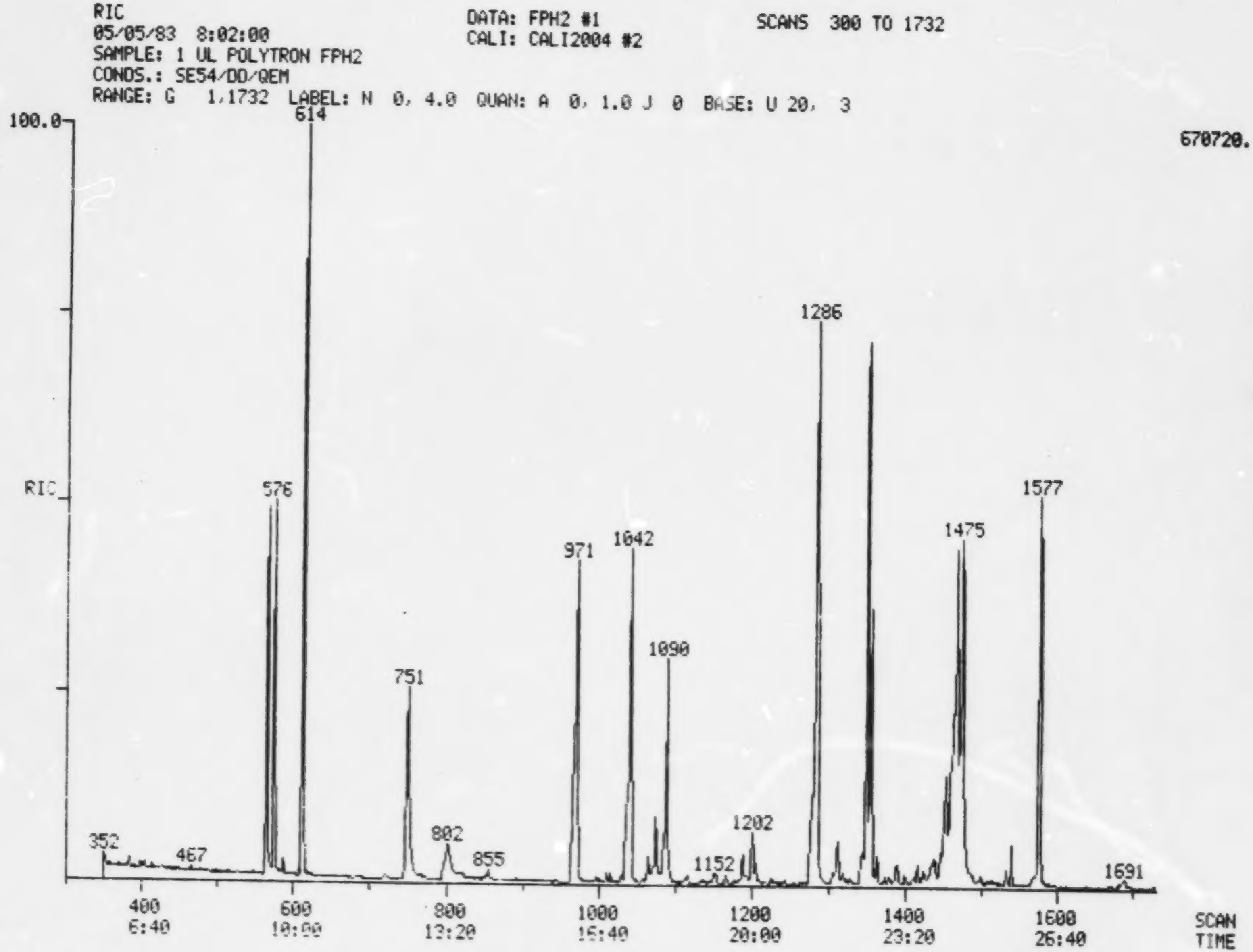


FIGURE - 2   RIC LOW LEVEL SPIKE

```
RIC                                    DATA: POLY2 #1261          SCANS  200 TO 1600
05/20/83 11:08:00                      CALI: CALI0505 #2
SAMPLE: 1 UL LOW LEVEL SPIKE POLYTRON HOMOGENIZATION
CONDS.: SE54/IS/QEM
RANGE: G   1,1600  LABEL: N  0, 4.0  QUAN: A  0, 1.0 J  0  BASE: U 20,  3
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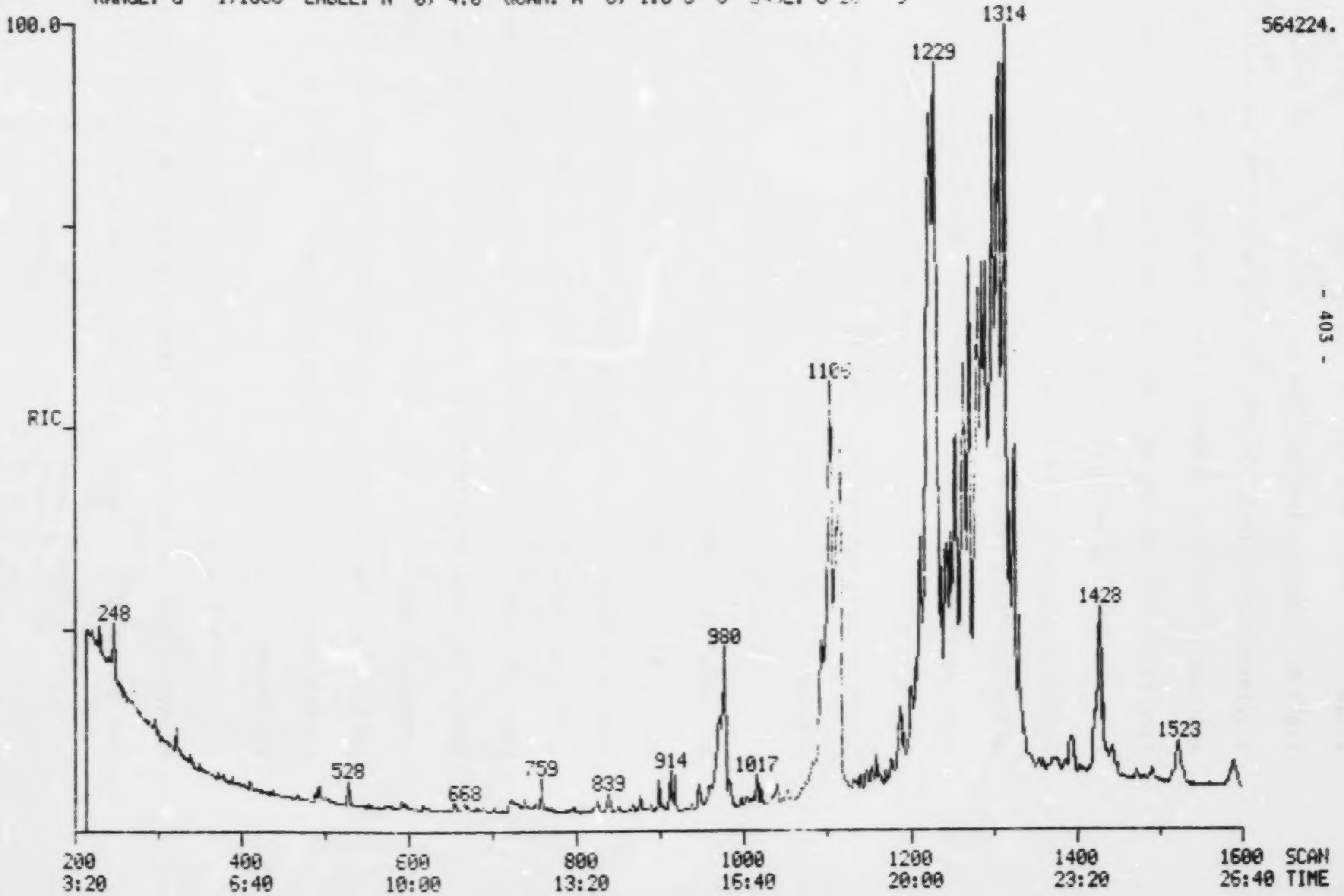

it's overall performance. GC/MS results from the acid digestion, steam distillation and polytron homogenization are listed in Table 6. Specific ion searches were also run for PAH and their alkylated derivatives, chlorinated furans and dioxins, chlordanes and each of the model compounds that were used in the fortification study but no traces of these compounds were found.

The low recovery of the labelled chlorophenol surrogate standard could be due to the low final extract volume (200 ul) or as a result of deuterium exchange as has recently been reported. Individual recoveries of this compound ranged from 2.7 to 76%. Further studies are necessary to identify the causes of this variability. The d_{10} anthracene gave consistent recoveries averaging 106% for all methodologies.

The GC/MS outputs of the polytron homogenization and acid digestion extracts contained rather large peaks that are mainly fatty acids and hydrocarbons. Each successive GC/MS run gave an increase in the total amount of material eluting from the glass capillary column. This increase is a carry over from the previous GC/MS run. Much of the interfering material can be removed by base saponification, however it could also affect compounds such as DDT. The steam distillation extracts produced relatively clean chromatograms but quantitated only 25% of the contaminants identified compared to the other extraction procedures.

PCB were observed in the extracts produced by all three methods. The concentration of total PCB was 0.60 ± 0.07 ug/g for the steam distillation 0.80 to .05 uglg for the polytron

TABLE 6

CONTAMINANTS IDENTIFIED IN REAL FISH HOMOGENATES BY GC/MS

(ng/gm)

CONTAMINANT	Mean Contaminant Concentration			Relative Recovery		
	Acid Digestion	Polytron Homogenization	Steam Distillation	Acid Digestion	Polytron Homogenization	Steam Distillation
Hexachlorobenzene	25.6	21.2	28.5	90	74	100
Pentachlorophenol	67.9	67.9	-	100	100	-
DDE	290	209	78.5	100	72	27
Octachlorostyrene	7.5	7.2	-	100	96	-
t-nonachlor	9.7	10.5	-	92	100	-
DDT	143	97.7	13.5	100	68	9
Mirex	37.0	33.9	10.2	100	92	28
Photomirex	8.7	8.8	2.5	99	100	28
Recovery (%)						
D-10 Anthracene	111	110	90.1	100	99	81
D-4 Chlorophenol	41	5.4	35	100	13	85

homogenization and 1.05 ± 0.06 ug/g for the acid digestion procedure.

CONCLUSIONS

The data obtained from the fortification studies and the analysis of naturally contaminated fish tissues show that the steam distillation technique is clearly inferior in the extraction and recovery of high boiling toxic organics from fish tissue samples. The acid digestion technique produced slightly higher recoveries of the chlorophenols, polycyclic aromatic hydrocarbons, volatile chlorinated hydrocarbons, and PCB in the fortification studies.

In addition, the acid digestion technique resulted in the higher contaminant concentrations for 75% of the compounds quantitated in the naturally contaminated tissues.

These results, along with the lower requirements in sample preparation time and equipment make the cold acid digestion technique the method of choice for the screening of Great Lakes sport fish for persistent and toxic organic compounds.

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The Development of a Freshwater Fish Test to Identify Aquatic Toxic Contaminants.

Ian R. Smith and V.E.O. Valli, 1983.

Department of Pathology, Ontario Veterinary College,
University of Guelph, Guelph, Ontario. N1G 2W1.

Supported by the Ontario Ministry of the Environment as
Provincial Lottery Project No. 64.

ABSTRACT

Determining the potential long and short term health effects of many of the chemicals being discovered in Ontario's receiving waters requires that new test approaches be developed. Gross genetic damage is a feature of many chemicals reacting with DNA, and in this study anaphase aberrations and micronuclei (indicative of chromosome damage) were assessed in fish embryo's. Brachydanio rerio (Zebrafish) embryo's were exposed to Ethyl methanesulphonate (EMS) for 24 hours. 1000 mg/L EMS is non-lethal to embryos over the 24 hour exposure period, however a reduction in developmental stage (growth) was seen in 100 and 1000 mg/L at 6 hours. A reduction in the mitotic index (total number of anaphases) was seen only in 1000 mg/L after 12 hours, and 10 mg/L after 24 hours. Exposure of Zebrafish embryo's to EMS resulted in an increase in anaphase aberration frequency, and in aberration severity, in as little as 6 hours when exposed to 100 mg/L or 24 hours when exposed to 1 mg/L. Micronuclei levels were insignificantly higher than control (due to wide variability) with 100 mg/L EMS, and further analysis of this approach's sensitivity is pending. It is apparent that both anaphase aberrations and micronuclei are sub-lethal phenomenon. A time and dose-dependant increase in embryo anaphase aberrations was present, and our experiments prove that this approach is as sensitive as more conventional approaches using adult fish. The impact of chemicals on somatic and germinal DNA is an important and growing area of study because these genotoxicants are linked to carcinogenesis, teratogenesis and mutagenesis. This test system provide's a means for detecting and assessing waterborne genotoxic chemicals at chemical levels below those affecting growth or survival.

INTRODUCTION

The increasing concern over aquatic contaminants with carcinogenic and mutagenic activity has prompted suggestions for using fish as sentinels (Beardmore et al., 1980; Alink, 1982). The monitoring of bioaccumulated chemicals in fish has been advocated (IJC, 1982), but not specifically for mutagenic and/or carcinogenic compounds. It may be more appropriate to look for the effects of mutagens/carcinogens rather than merely determining their presence. The potential impacts of aquatic mutagenic/carcinogenic compounds are very wide ranging, for both humans and the inhabitants of the water bodies affected (for review's see Alink, 1980; Klingerman, 1982). The most straight-forward approach is to determine that a change in DNA structure (mutation) has occured. The mounting evidence that somatic mutations may be a causative factor in carcinogenesis (Strauss, 1981) and that most carcinogens are mutagens (Ames and Hooper, 1978; Hart et al., 1977) has led investigators to study mutations arising in fish.

Work in detecting elevations in chromosome damage (Stromberg et al., 1981; Alink et al., 1980; Prein et al., 1978; Hooftman and Vink, 1981; Longwell and Hughes, 1980; Longwell 1981) has demonstrated that environmental contaminants can and do induce measurable chromosome damage in fish. The isolation of causative agents (mutagens and clastogens) requires that laboratory studies be performed either with the chemicals isolated from or preferably with the industrial effluents contributing to the area. While laboratory test systems using adult fish have been developed, chemical concentrations inducing significant effects normally do so only at very high or extended levels. Fish cell lines have been proposed (Beardmore et al., 1980) for increased sensitivity in screening aquatic contaminants, however whole organism assays would be preferable. The development and substantiation of an assay applicable to both laboratory and field work is the goal of this research program.

A variety of endpoints have been utilized in the past to assess the effects of mutagenic chemicals on fish, inluding sister-chromatid exchange (SCE), micronuclei (MN), and chromosome aberrations measured at both metaphase (MA) and anaphase (AA). While the SCE approach has gained wide acceptance, it requires that organisms be treated on two occasions with mutagenic chemicals, thus artificially increasing the background levels of mutation. The analysis of MA similarly requries colchicine, itself mutagenic, and both procedures are painstaking and time-consuming. The analysis of MN and AA do not utilize additional chemicals, do not increase the background rate of mutation, and appear's to *be the approach of choice. The sensitivity of any selected approach is of utmost importance, however little information is available to determine the relative sensitivity of the various endpoints, and this must then be explored before choosing a test.*

The types of organisms which can be utilized is greatly affected by the chosen endpoint. Both the SCE approach and MA analysis require that organisms with a low number of large chromosomes be used, and this restricts the number of potential test organisms, especially for field work. While the mudminnow (Umbra limi) has proven to be useful in the field and laboratory, it is difficult to obtain large numbers of these fish. Because they must be obtained from the wild, mudminnow's are subject to background chemical burdens. The killifish (Nothobranchius rachowi) is now being utilized for laboratory work in Europe, it having the necessary chromosome complement for such studies, but it is expensive to raise and maintain because it has a short lifespan, is a limited spawner, and its eggs require a diapause to develop properly. An approach utilizing MN and/or AA would eliminate the neccesity for a low chromosome complement, be applicable to all species of fish, and would allow the use of the same approach in both the field and laboratory.

The search for a sensitive assay in aquatic toxicology has led to the use of embryo-larval stages, their sensitivity being comparable to that of chronic or life-cycle tests with juvenile and adult fish (McKim 1977; Macek and Sleight, 1977). The use of embryos to test pure compounds and effluents for mutagenicity may provide a sensitive whole organism test system in both the field and the laboratory. Their use in the field and laboratory has been investigated somewhat (Longwell and Hughes, 1980; Longwell, 1981; Hose et al., 1983) and they appear to be quite sensitive. The use of tropical fish embryo's in the laboratory has several advantages over cool or cold water fish: small eggs with relatively little yolk requiring higher temperatures allow for rapid development and a high number of mitotic figures; eggs can be exposed in small, static, non-aerated systems for a considerable period of time, and the fish are inexpensive and easy to raise and breed. This study provides evidence of fish embryo's response to a proven mutagen to provide information on the sensitivity and background values of an approach using AA and MN.

MATERIALS AND METHODS

Commercially obtained Brachydanio rerio (Zebrafish) adults 1.5 to 2 years old were held in activated carbon dechlorinated city of Toronto water in a 100 L glass aquarium with a 12/12 photoperiod and some natural lighting. The adults were fed Tetramin (TetraWerke Dr.rer.nat.Ulrich Baensch GmbH) staple flake food and frozen brine shrimp twice daily, and pairs were spawned in 10 L aquaria at 25 degrees C with aeration.

Beginning at the 2-64 cell stage, embryos were exposed to 20 ml test solution in 40 ml glass petri dishes at 25 degrees C. Ethyl methanesulphonate (EMS; Sigma) was diluted with dechlorinated water. Four separate tests were run, one for each concentration, with both control and exposed embryos (30 per concentration) fixed in buffered formalin at 6, 12 and 24 hours. Chemical concentrations tested were 1, 10, 100 and 1000 mg/L EMS.

Ten embryos were dechorionated, treated with 50% acetic acid for 15 minutes (after Longwell and Hughes, 1980) and then stained (5 minutes) and squashed in a solution of freshly filtered aceto-orcein containing 5% propionic acid. The edges of the slide coverslips were sealed to make a semi-permanent preparation. Randomly numbered slides from "normal" embryos were examined for aberrations in twenty late anaphase's per embryo (AA) at 1000X total magnification. Aberrations scored were principally chromatin bridges, acentric and attached fragments, lagging chromosomes and multipolar figures (Nichols et al., 1977;Kocan et al., 1982). Single chromatin bridges are indicative of an incomplete or asymmetrical exchange of DNA, leading to a thin strand of DNA being stretched between the dividing groups. Breaks in the chromatids or chromosomes are visible as unattached fragments at the equator (acentrics) or attached fragments which are joined to a group by a thin thread of chromatin.

Lagging chromosomes and multiple bridges are thought to be indicative of cellular toxicity resulting in sticky chromosomes (Longwell, 1978). Laggard chromosomes or attached and acentric fragments, which are left outside of the nucleus during the completion of division, form micronuclei, visible as small dark-staining bodies up to one-tenth the size of the normal nucleus. These were scored in the yolk-sac cells visible at 24 hours only, as insufficient numbers of yolk-sac cells are present at 6 or 12 hours. A normal embryo was classified as being normally developed grossly, possessing at least 10 anaphases microscopically and being without an excess of amorphous areas, consisting of denatured chromatin, a characteristic of moribundity (Longwell and Hughes, 1980). A limited number of amorphous areas and pyknotic cells was considered normal, due to the resorbtion of embryonic tissues which were no longer necessary.

Data for up to 10 embryos from each time and concentration were analyzed for survival, total number of anaphases, number of anaphases with aberrations in the first 20 examined, and a developmental index, determined by assigning a number (based on Hisoaka and Battle (1957)) to each embryo and determining the average value. Micronuclei were analyzed at 24 hours only. Statistical analysis consisted of the Student's t-test at a 0.05 level of significance and ANOVA using the University of Guelph's APL Statistical library.

RESULTS

Control values

The design of this experiment facilitated a comparison of survival, AA and MN background frequency, developmental stage and number of anaphases for different groups of embryo's. The control fish featured a survival ranging from 96% (1000 mg/L) to 53% (1 and 10 mg/L), at 24 hours. All embryo's had 2 to 64 cells when the experiments were started, an age at which it is very difficult to remove the abnormally developing embryo's which are unlikely to survive.

The growth (developmental index) of the control groups was variable at all ages. To eliminate control variability, results for growth parameters are expressed as percentages of control values. The embryo's were mid-gastrulas at 6 hours, early embryo at 12 and mid-embryo at 24, corresponding to stages 15, 18 and 20 respectivly under the classification of Hisaoka and Battle (1958). The number of late anaphases was similar, only the 100 mg/L controls at 12 and 24 hours being different from another control group (1 mg/L) at the same age. Wide variability in the total number of anaphases was present, however the total number of anaphases in control embryos declined insignificantly from 31.2 at 6 hours to 28.1 at 12 and 27.8 at 24 hours.

AA background rates varied from 1.6/20 to 0.4/20 in groups of different parentage at different ages. The average background rate at 24 hours of age was 0.75/20, 0.9/20 at 12 hours and 1.53/20 at 6 hours of age. No relationship between control survival and anaphase aberrations was seen between groups, however the decrease from 1.53/20 at 6 hours to 0.9/20 AA's at 12 hours corresponded with the general finding that mortalites were first evident at 12 hours. In only one instance was any control anaphase aberration rate significantly different at the same stage from another (1000 mg/L control versus 10 mg/L control at 6 hours). Background rates of yolk-sac MN at 24 hours of age varied from 0.32% to 1.6%. MN rates appeared somewhat to be related to survival, as the 1 and 10 mg/L controls, which had 50% control survival, had the highest MN values (1.6% and 0.87% respectivly). None of the control MN frequencies were significantly different. Based on the aforementioned findings, all control embryos regardless of parentage featured similar background levels of AA's, MN and growth indicators while survival variability appeared to be due to parental differences in two groups.

Survival:treated embryo's

The survival of EMS treated embryo's was only significantly different from control in one tested group, the 100 mg/L group at 24 hours, a reduction by 50% (Figure 1). This was an anomaly, as no significant difference in survival was evident between control and treated groups at 12 hours in 100 mg/L EMS, or at any time in 1000 mg/L. Subsequent testing with EMS has found that the 24 hour LC50 is in excess of 1000 mg/L and repeated experiments with both embryo's and juveniles has yielded little 24 hour mortality in EMS concentrations up to 1000 mg/L. Analysis of the trends in survival of treated embryo's versus controls (Figure 1) reveals that at all times 1 and 10 mg/L EMS had no significant effect. 1000 mg/L and 100 mg/L EMS consistently (5 of 6 concentration/time combinations) yielded relative survival only slightly less that 100% (an average of 87% survival). The onset of mortality in both control and exposed fish was generally at 12 hours, very little mortality being seen at 6 hours. EMS is non-lethal at concentrations up to 1000 mg/L for 24 hours.

Growth and division rate:treated embryo's

The greatest effect on growth was apparently felt at 6 hours (Figure's 2A,B) as the 1000 and 100 mg/L concentration's reduced the developmental stage to 80% and 90% of control respectivly. This delay apparently was temporary as by 12 hours all concentrations were reduced to only 95 to 98% of control. At 24 hours an apparent concentration dependant depression of developmental stage was again visible (Figure 2A), and this reduction of growth by 5 and 10% in 100 and 1000 mg/L was significant. It appears that the gastrula stage (6 hours) and mid-embryo (24 hours) stages are somewhat more susceptable than the early embryos (12 hours) to the toxicant effects of EMS (Figure 2B). With the exception of 100 and 1000 mg/L at 6 hours, it was felt that the reduction in growth (developmental stage) was of little consequence to the embryo.

As a second measure of growth rate (mitotic index) the total number of anaphases per embryo was examined (Figure 3). The effect of EMS appears to be a concentration-dependant reduction in the number of anaphases below control levels, in 10 mg/L EMS and above at 24 hours and in 1000 mg/L alone at 12 hours. The pattern at 6 hours is highly variable. The effect seen in the 100 mg/L EMS embryo's at 6 hours coincides with the reduction in devlopmental stage seen in the sensitive gastrula stage (Figure 2). The absence of an effect in 1000 mg/L is unexplained. It may have been that these embryo's had their growth slowed so much as to not have

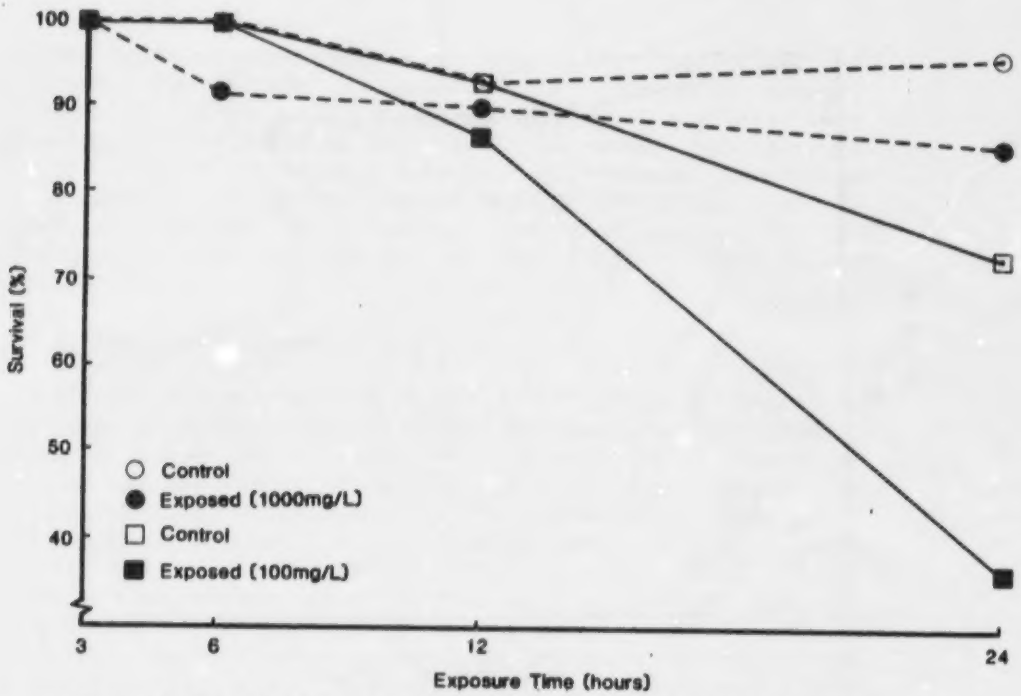

Figure 1A : Survival of Brachydanio rerio embryos exposed to 100 and 1000 mg/L Ethyl methanesulphonate and unexposed paired controls for up to 24 hours.

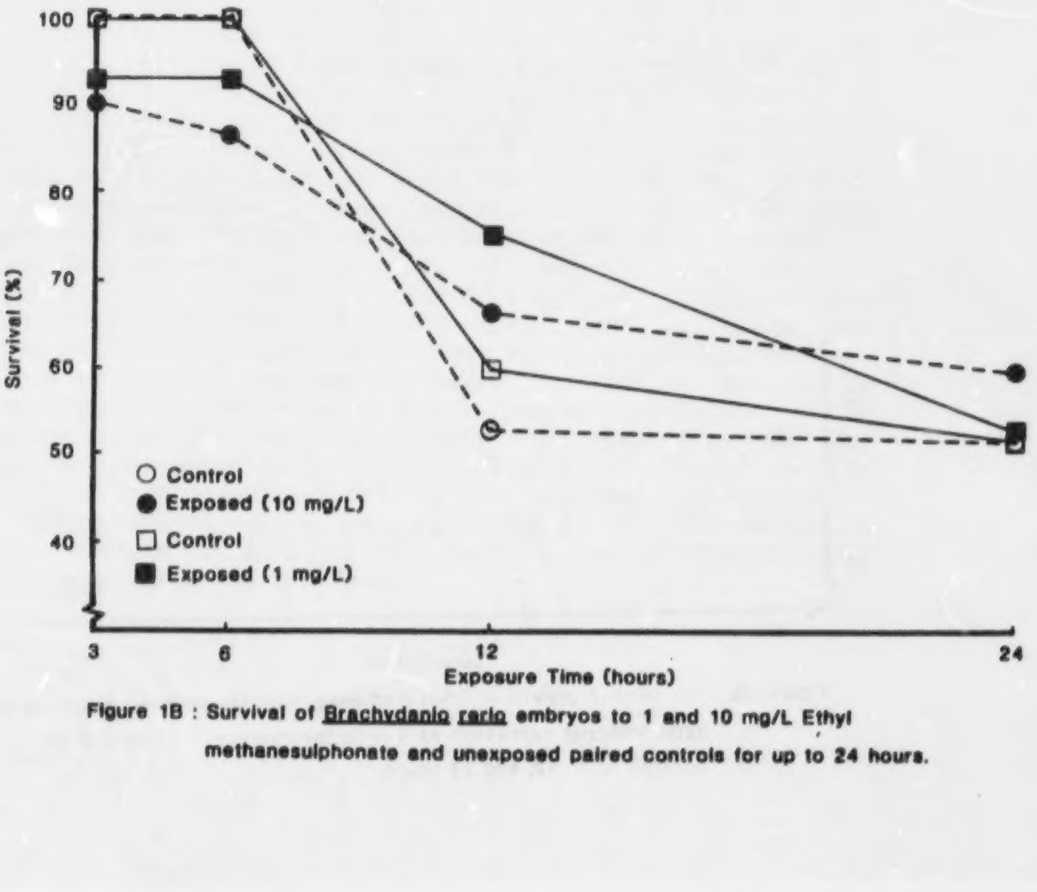

Figure 1B : Survival of Brachydanio rerio embryos to 1 and 10 mg/L Ethyl methanesulphonate and unexposed paired controls for up to 24 hours.

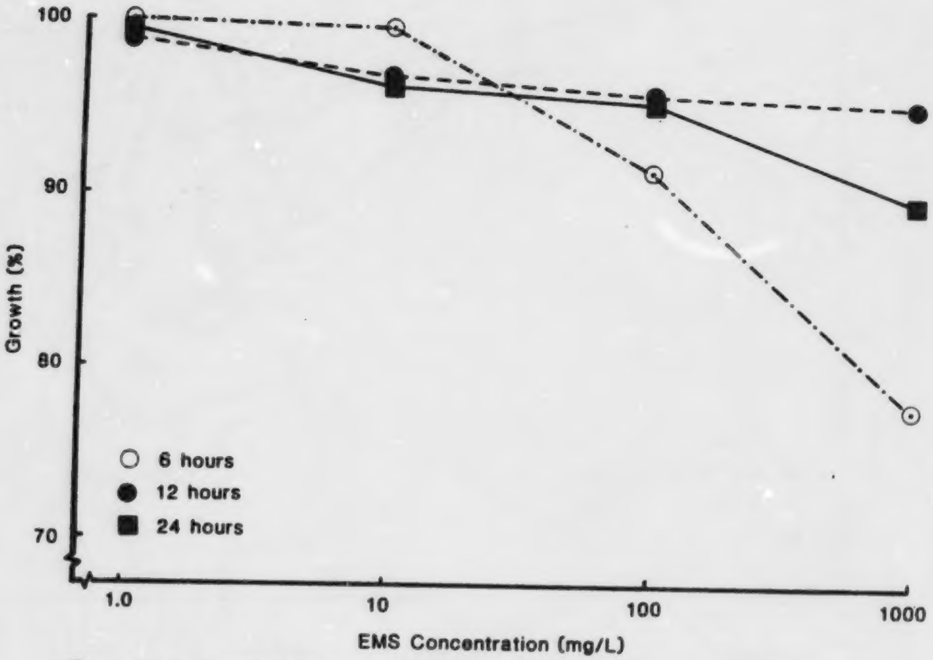

Figure 2A : Growth of Ethyl methanesulphonate exposed Brachydanio rerio embryos after 6, 12 and 24 hours. Growth is expressed as the ratio (percent) of the exposed embryos quantitative developmental index to the controls (unexposed embryos) thus indicating the relative reduction of developmental stage only.

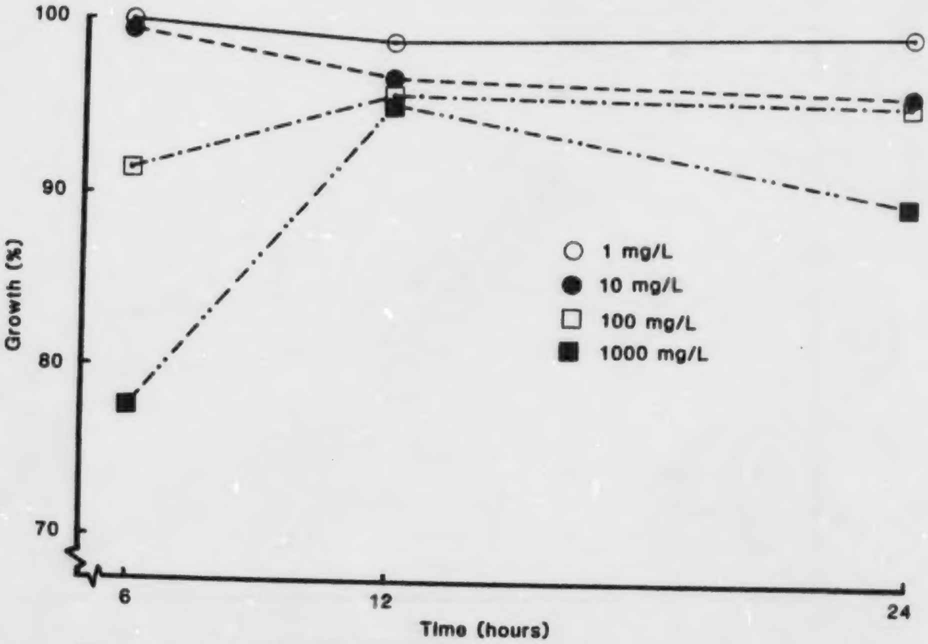

Figure 2B : The relative growth of Ethyl methanesulphonate exposed Brachydanio rerio embryos expressed as a ratio (percentage) to their paired controls at 6, 12, and 24 hours.

reached critical gastrulation as yet. The "piling-up" of anaphases (due to the accumulation of chromosome damage) during critical gastrulation in embryo's which may have then died before they could be analyzed at 12 hours may account for the wide variability at 6 hours. Significant reductions below control levels were present only in the 10 mg/L at 24 hours, and 1000 mg/L at 12 hours. The wide variability in values of many groups prohibited any further findings of significance.

Micronuclei:treated embryo's

Micronuclei analysis was carried out only at 24 hours in the large polygonal yolk-sac cells because the cytoplasmic volume of these cells was quite large. An increase in micronuclei was seen in both 1000 and 100 mg/L EMS at 24 hours, levels of 4.5/100 and 2.2/100 respectivly being 14.2 and 5.1 times control. A very wide variability was present in yolk-sac micronuclei (often 150%) preventing findings of significance, however when the frequency of treated embryo's micronuclei is expressed as a percentage of control (Figure 4) a very clear trend is evident, MN increasing markedly with concentration. A semi-quantitative increase with concentration was seen in the number of pyknotic (dead) cells, not unlike micronuclei.

Anaphase Aberrations:treated embryo's

The effect of EMS on the total number of chromosomal abnormalities at anaphase was quite remarkable in its severity and speed of onset (Figures 5 and 6). In the embryos exposed for 6 hours the 100 mg/L embryo's had a significant increase of 2.5 times the AA frequency of control, while the 1000 mg/L embryo's had a significant increase to a level 7 times control. By 12 hours the 100 mg/L embryo's AA frequency was 9 times control and the 1000 mg/L embryo's were 10 times control. At 24 hours, the doses 10, 100, and 1000 mg/L had induced a significant increase in AA frequency to levels 2.3, 10 and 29 times control respectivly. The concentration of 1 mg/L EMS increased the AA frequency by a factor of 60% above controls, which was statistically insignificant. Thus a significant increase was seen in 100 mg/L and above as early as 6 hours, and in 10 mg/L and above at 24 hours versus paired controls. At 24 hours the AA frequency was significantly related to the log of the concentration ($p < 0.001$, $r2 = 78.7$). Based on the lack of control variability, it was considered appropriate to combine control values, this resulting in the 60% elevation observed in 1 mg/L at 24 hours becoming significantly different from control ($n=40$).

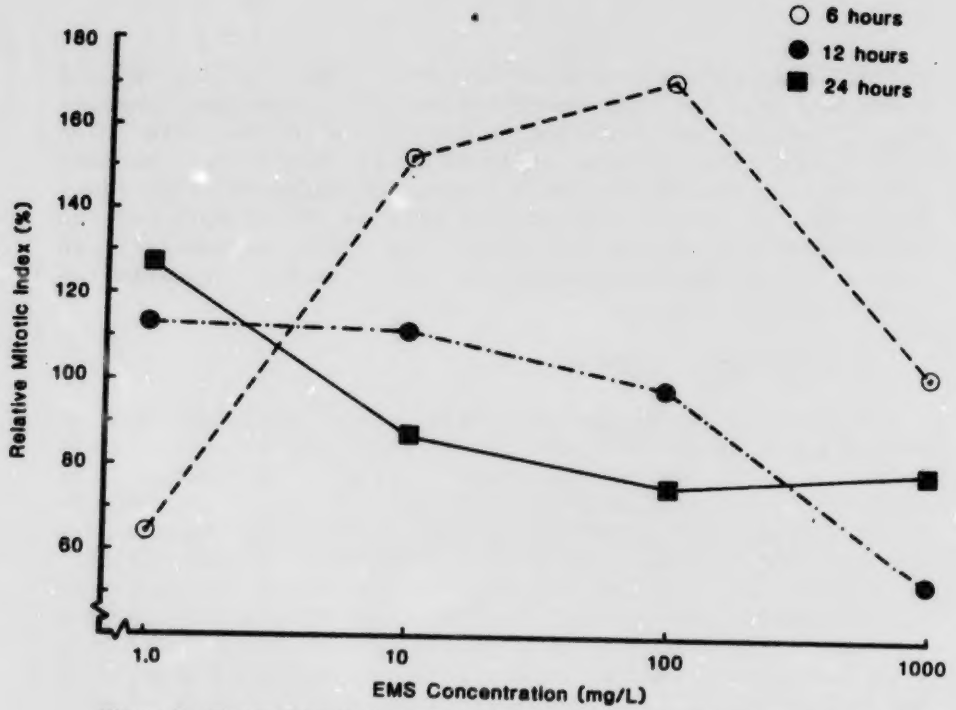

Figure 3 : Fluctuations in the relative mitotic index (the ratio (%) of the no. of anaphases in exposed embryos versus the no. in control) in *Brachydanio rerio* embryos exposed to Ethyl methanesulphonate for 6, 12, and 24 hours.

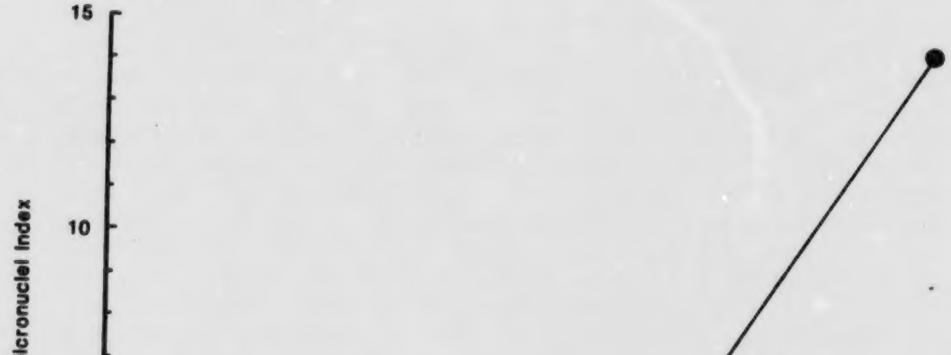

Figure 4 : The influence of Ethyl methanesulphonate on the abundance of yolk-sac micronuclei in *Brachydanio rerio* embryos exposed for 24 hours. The number of micronuclei in the exposed embryos divided by the number in the control embryos forms the Relative Micronuclei Index.

An analysis of the change in AA rate over time (Figure 6) reveals that the EMS concentrations of 1, 10 and 100 mg/L induce generally more aberrant anaphases with time, up to 24 hours. The 1000 mg/L EMS appears to have levelled off at 12 hours, with 77% of the anaphases present having aberrations.

The types of defects induced by EMS were primarily attached and acentric fragments, lagging chromosomes and chromatid bridges (single), accounting for 94% of the observed abnormalities at 24 hours (Table 1). The pattern is quite different at 6 and 12 hours as at these times multiple abnormalities (generally a combination of multiple bridges or bridges with another type of defect) were the largest single type of damage, followed by fragments, bridges and lagging chromosomes. The elevated incidence of multiple defects at 6 and 12 hours may reflect increased sensitivity at the cell level to the toxic effects of EMS.

The ratio of several of the types of damage to each other yields some insight into the action of EMS. Comparison of the ratio of either of the two types of fragments (acentric and attached) to lagging chromosomes in all times reveals that the ratio increases markedly with concentration. This is also the case when comparing the ratio of fragments to bridges in the 6 and 24 hour exposed embryo's, and would hold true in the 12 hour embryo's except for the large increase of multiple defects, reducing the apparent incidence of single bridges artificially, as most multiple defects included bridges. It would appear that attached and acentric fragments are the primary damage caused by EMS, the bridges and lagging chromosomes being secondary damage.

Table 1A: The anaphase abnormalities in control and exposed embryos at 6 hours after the initiation of the experiment. Control embryo's are pooled at each time. All values are expressed relative to the number of anaphases examined for defects, normally 20 in each embryo.

Dose	Number of Embryo's	% Normal	% Attached Fragments	% Acentric Fragments	% Chromatin Bridges	% Lagging Chromosomes	% Side-arm Bridges	% Multipolar Figures	% Multiple Defects	Others
0	25	94.5	0.0	0.5	1.8	2.7	0.0	0.0	0.4	0.0
1	6	96.6	0.0	0.8	0.8	1.7	0.0	0.0	0.0	0.0
10	7	94.3	0.0	0.0	0.7	3.5	0.0	0.0	1.4	0.0
100	10	84.0	0.0	1.5	5.5	4.5	0.0	0.0	4.5	0.0
1000	10	46.3	2.6	12.1	11.1	13.7	0.0	0.5	14.2	0.0

Table 1B: As for Table 1A except that values are for embryo's after 12 hours.

Dose	Number of Embryo's	% Normal	% Attached Fragments	% Acentric Fragments	% Chromatin Bridges	% Lagging Chromosomes	% Side-arm Bridges	% Multipolar Figures	% Multiple Defects	Others
0	37	96.5	0.0	0.1	0.6	2.7	0.0	0.0	0.0	0.0
1	10	97.9	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0
10	10	95.4	0.5	0.0	0.5	3.5	0.0	0.0	0.0	0.0
100	10	79.0	0.5	7.5	3.5	7.5	0.0	0.5	1.5	0.0
1000	7	22.5	0.9	13.5	2.7	14.4	0.0	0.0	46.8	0.0

Table 1C: As for Tables 1A and 1B except that values are for embryos after 24 hours.

Dose	Number of Embryo's	% Normal	% Attached Fragments	% Acentric Fragments	% Chromatin Bridges	% Lagging Chromosomes	% Side-arm Bridges	% Multipolar Figures	% Multiple Defects	Others
0	40	95.3	0.4	1.0	2.1	0.6	0.1	0.0	0.0	0.4
1	10	91.7	0.8	1.6	2.7	2.4	0.0	0.0	0.0	0.8
10	10	90.4	0.5	0.9	2.7	2.7	0.5	0.9	0.5	0.9
100	10	61.9	2.3	10.0	5.4	14.6	0.7	1.9	2.7	0.4
1000	10	33.5	7.9	21.9	15.9	16.5	0.0	1.2	3.0	0.0

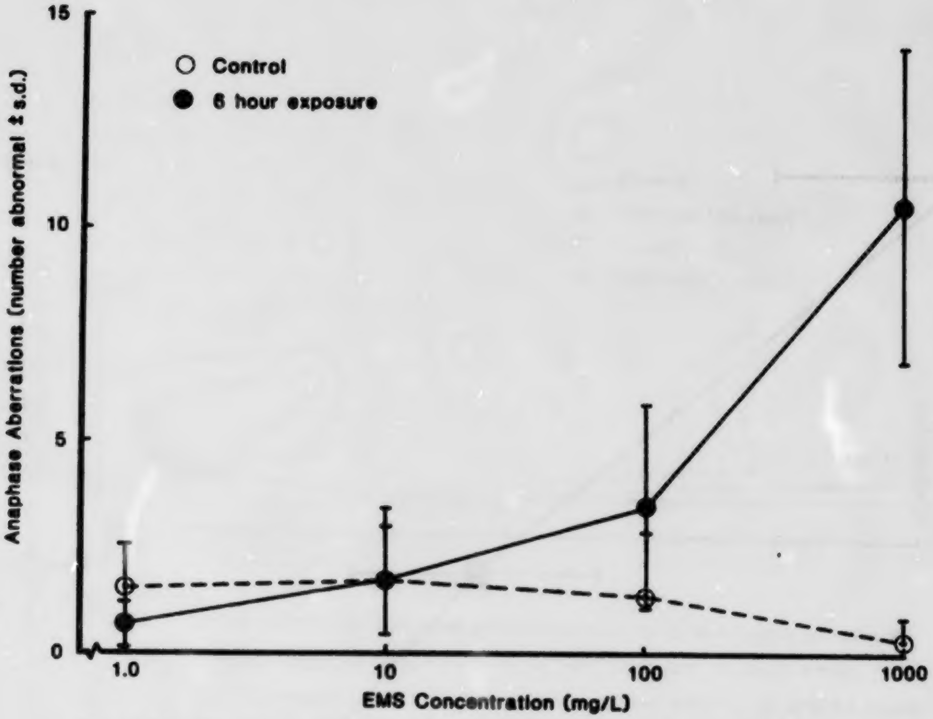

Figure 5A : The genotoxicity of Ethyl methanesulphonate in Brachydanio rerio embryos exposed for 6 hours. Genotoxicity is measured as the number of A.A.'s in 20 anaphases examined per embryo.

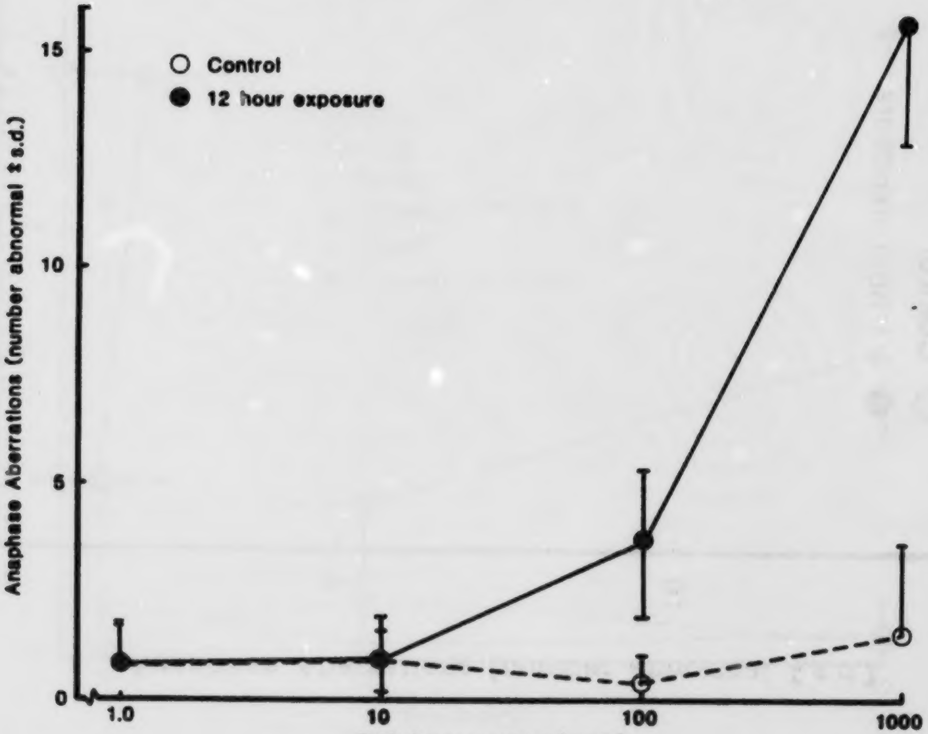

Figure 5B : The genotoxicity of Ethyl methanesulphonate in Brachydanio rerio embryos exposed for 12 hours.

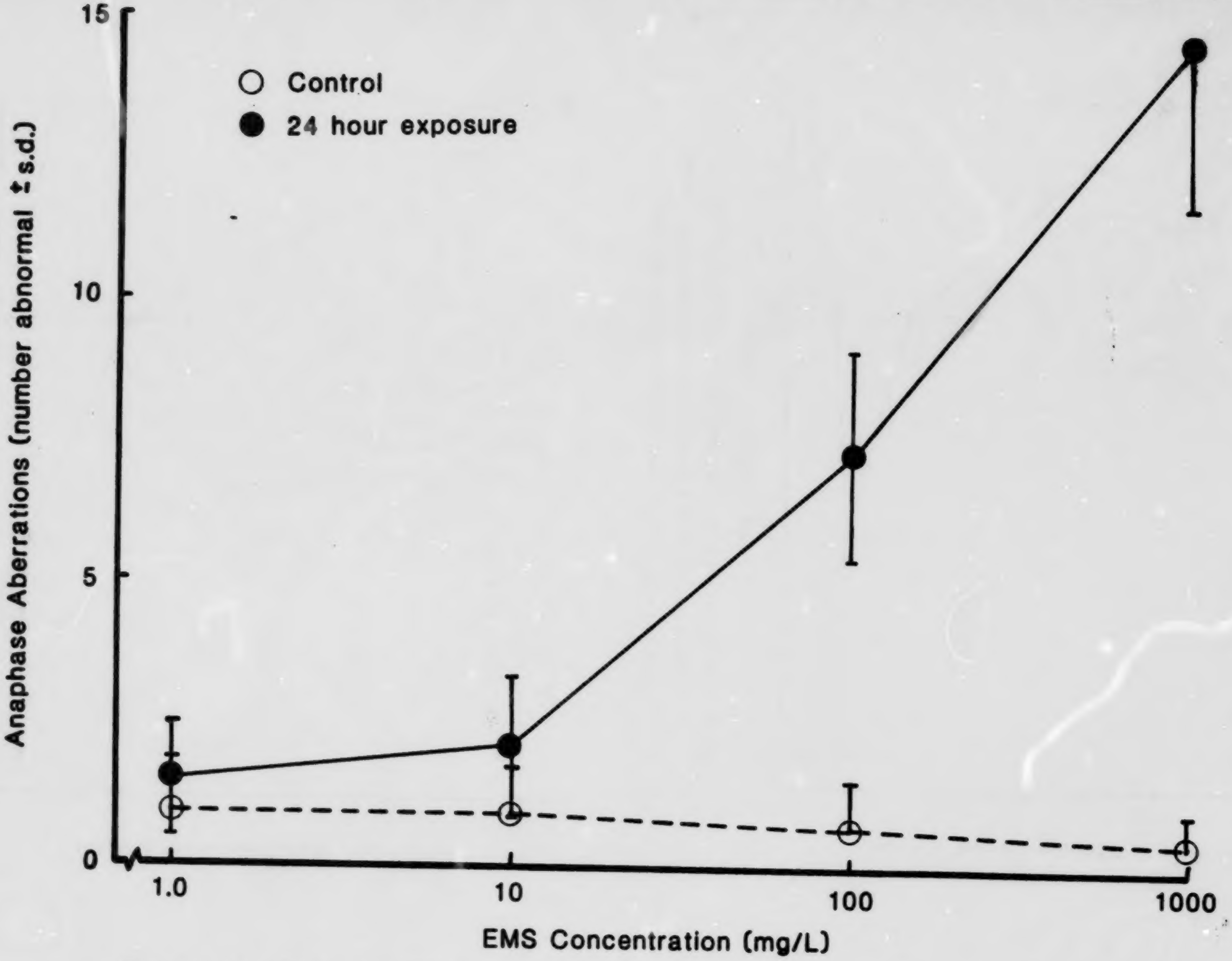

Figure 5C : The genotoxicity of Ethyl methanesulphonate in *Brachydanio rerio* embryos exposed for 24 hours.

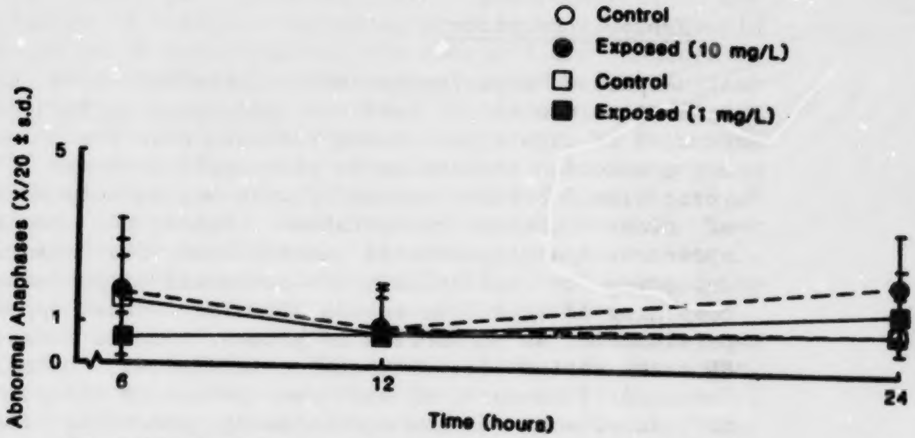

Figure 6A : The number of abnormal anaphases (A.A.'s/20 anaphases examined) in Brachydanio rerio embryos exposed to 1 and 10 mg/L Ethyl methanesulphonate and their paired controls after 6, 12 and 24 hours.

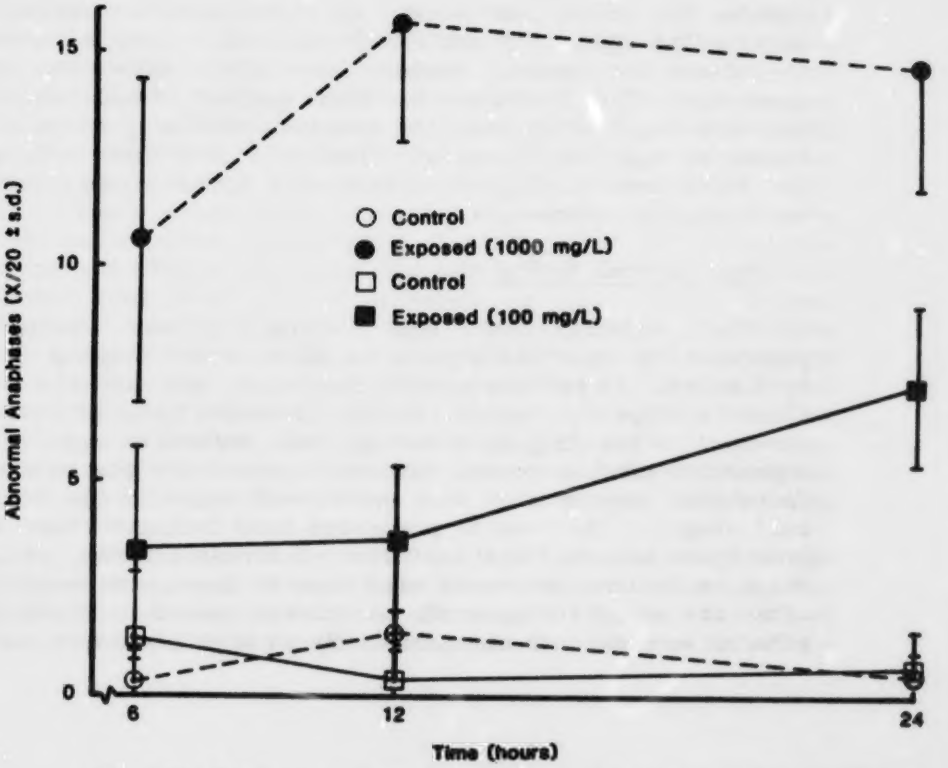

Figure 6B : The number of abnormal anaphases (A.A.'s/20 anaphases examined) in Brachydanio rerio embryos exposed to 100 and 1000 mg/L Ethyl methanesulphonate and their paired controls after 6, 12 and 24 hours.

DISCUSSION

Embryo Production

Repeated experiments with Zebrafish have highlighted several points which must be addressed prior to further studies of this type. Zebrafish mature at 4 months of age, egg production continuing to rise up to 2 years of age, at which time increased variability in egg quality makes the use of older females undesirable. Zebrafish are fractional spawners, spawning several times a week for several weeks, depending on nutrition (of paramount importance). The combining of eggs from several females would appear to be permissable, as parameters of growth, mitotic index, AA and MN rates varied little between different control groups. Variability in survival was due primarily to the females influence, some females consistently producing spawns with 95% survival, other's consistently producing 5% survival. By spawning a female several times and recording survival, it is an easy task to accumulate several females with excellent spawning success. The availability of a large number of healthy eggs continuously for up to 3 weeks at a time can then be assured.

Over-ripeness appear's to be a problem if females are isolated for 15 days to a month (Niimi and La Ham, 1974; Laale, 1977) however our laboratory has successfully isolated females for up to two weeks with no apparent effect on survival of eggs. The end result of isolation is a spawn 2 to 3 times the regular number immediately upon the first spawning. This technique has been applied in our laboratory by isolating females over the weekend, resulting in a large number of eggs for bioassay's Tuesday or Wednesday. This has the added benefit of giving the female a chance to increase her energy reserves.

Experimental Method

The majority of eggs dying without completing gastrulation had visible division defects or lagging growth by 6 hours. By waiting until the eggs are in the early blastula stage (6 hours) it is possible to achieve 95% survival to hatching by removing these defective eggs. This approach begin's exposure before the sensitive gastrula stage (note that exposures in this experiment began in the 2 to 64 cell stage). The results presented here indicate that while growth was an excellent indicator of stress at the gastrula stage, mutations increased with time in lower concentrations after the sensitive gastrula period was passed. Significant effects were seen in mutations only in high concentrations at the gastrula stage. While sensitivity to maternally transferred mutagens (yolk) or paternal effects (ie. dominant lethality) may be greatest at gastrulation, the administration of mutagens beginning at 6 hours of age would appear to be quite acceptable if the embryo's are examined at 24 hours. Published studies (Hose et al., 1983) with English sole (Parophyrs vetulus) embryo's found significant cytogenetic effects at the gastrula stage. No time dependancy was investigated in these studies, and exposures utilized Benzo-a-pyrene administered at 11.5oC (beginning at the blastula cap stage) sampling after an unknown time (propably in excess of 48 hours at this low temperature). This work substantiates the possibility of beginning exposures at blastula, rather than at fertilization.

A note of interest regarding the use of Zebrafish as mutagen test organisms is the small size of the eggs, facilitating large numbers of embryo's in a small amount of test chemical (we have grown 50 embryo's in 20 ml. test solution for up to 4 days with no apparent ill effects) without replacement. Small amounts of test chemical can be used with eggs, as opposed to experiments with adult fish, requiring large amounts of test chemical.

Health effects

The absence of EMS induced mortality in this experiment was to be expected. The 96 hour LC50 of EMS with juvenile Zebrafish is approximatly 750 mg/L, while 250 mg/L is completly non-lethal, although 72 hours in 1000 mg/L induced 100% embryo lethality in subsequent assays. A variety of authors experimenting with EMS and adult fish have reported no mortality in EMS levels up to 200 mg/L for several weeks (Hooftman and Vink, 1981; Hooftman and Raat, 1982; van der Hoeven et al., 1982). The absence of mortality in 1000 mg/L over 24 hours means high chemical levels and a short exposure period can be used, providing a starting point for definitive testing. A similar approach has been taken for the induction of tumours in trout (embryo's; Wales et al., 1978; Wales, 1979; Hendricks et al., 1980) and tropical fish (juveniles; Schultz and Schultz, 1982a,b) by applying high concentrations over a period too short to induce mortality. The relative effects of short time/high concentration versus long time/low concentration has yet to be investigated. For screening purposes a 24 hour exposure is very useful, and little difficulty in preparing slides for microscopic examination was encountered at this age.

The growth reduction evident at 6 and 24 hours may be due to tissue damage, cytotoxicity or some other effect. The periods of apparent sensitivity correspond to the onset of gastrulation and organogenesis respectivly. Damage to early "primitive" tissues could severly delay further development, other cell types being forced to differentiate, replacing the damaged ones. Subsequent studies performed with EMS have shown the growth reduction caused by 24 hours exposure to EMS is not recovered, even if the embryo's are removed to clean water, for the period up to hatching (4 days). The use of the number of anaphases as a mitotic index shows a tissue-level growth reduction at 12 hours in 1000 mg/L EMS and above 10 mg/L at 24 hours. This indicates that the ability of individual cells to divide normally corresponds directly to the growth delay seen in 24 hours as reduced developmental stages. The absence of a growth delay at 12 hours probably reflects the death of moribund embryo's between 6 and 12 hours.

A decrease in spontaneous (background) AA frequency over time was seen in this study corresponding to the onset of mortality in all groups between 6 and 12 hours. Similarly, a decrease in AA's after gastrulation in Atlantic mackeral (Scomber scombrus) corresponded with an increase in the percentage of non-moribund and live embryo's sampled (Longwell, 1978). Both studies support the contention that many embryo's or cells with abnormal divisions don't complete gastrulation (A.C. Longwell, pers. comm.). The level of abnormal anaphases in control fish (approximatly 7.6%,4.5% and 3.7% at 6,12 and 24 hours of age) was similar to those found for English sole (4.3%; Hose et al., 1982) and mackeral (5.9% across a range of ocean samples; Longwell and Hughes, 1980). These values are much lower than the 12% found in a Rainbow trout (Salmo gairdneri) gonad cell line (Kocan et al., 1982).

The yolk-sac micronuclei found in this study (the numbers were insignificantly higher than control) supports the use of this approach with fish. Hooftman and Raat (1982) have been able to induce a statistically significant dose-dependant increase in micronuclei in adult fish with EMS. Other authors using fish and sea-urchin embryo's (Hose et al., 1982,1983), while finding an increase apparently were unable to generate a dose response or a significantly increased micronucleation rate. A possible problem with micronucleus use in embryo's is related to the natural process of apoptosis taking place (see Wyllie et al., 1980 for a review) which results in bodies very similar to micronuclei being phagocytozed by surrounding tissues. If a tissue is undergoing normal "degeneration" and subsequent apoptosis (embryo's would for example remove phylogenetic vestiges and metamorphose through apoptosis) it would be difficult to seperate this from the basophilic necrotic nuclear debris resulting from cytotoxicity. It has been suggested (see the review by Wyllie) that apoptosis is the mechanism by which proliferating tissues eliminate cells with genetic *abnormalities (mutations); regardless micronuclei may be confused with apoptotic bodies either "normal" or "mutant" in origin, or phagocytozed necrotic debris. Further investigations are planned to further assess the relative efficiency of the micronucleus test in embryo's, and its relation to apoptosis.*

The usefullness of Zebrafish embryo's for assessing waterborne mutagens appears to be very good. It is apparent that both a time and concentration dependancy are present in AA analysis. In lower concentrations (1 to 100 mg/L) the amount of damage increases with time until 24 hours, perhaps continuing to rise until some plateau is reached. This plateau was reached in 1000 mg/L after 12 hours. A longer exposure may make this test more sensitive to lower chemical levels for two reasons. Bio-accumulated chemical burdens will increase with time until a steady state is reached; and secondly, some types of damage require several rounds of replication before they become apparent, or are "fixed", while others dissapear through cell death. While micronuclei abundance was too variable for statistical significance to be found in this study, a study with the mudminnow (Hooftman and Raat, 1982) found an increase in peripheral blood micronuclei with time. This increase was due to the two factors previously described, the rise in numbers of micronucleated erythrocytes occuring because this damage type is quite persistent. In contrast to MN, the examination of AA's represents a single point-in-time measure of damage, damage which becomes invisible when mitosis is aborted, or becomes fixed, either as micronuclei, or some viable mutation in the daughter cell.

The absolute sensitivity of this test approach is excellent, based on the findings of a significant increase in 24 hours of AA's in 10 mg/L EMS with paired (n=10) controls, or in 1 mg/L EMS with grouped (n=40) controls. This level of sensitivity can be compared to published work. Studies with other species of adult fish have detected significant increases in genetic damage at levels of 8 mg/L EMS (killifish) after a 48 hour exposure (Hooftman, 1981), 40 mg/L (mudminnow) for 48 hours (Hooftman and Vink, 1981) and 50 hours (killifish) or 120 hours (mudminnow) in 24 mg/L EMS (van der Hoevan et al., 1982). In these cases, measurments of genetic damage were made in classically accepted fish species, with a small number of large chromosomes, measuring damage as aberrations or SCE's at metaphase. While it has been suggested (Klingerman, 1982) that these approaches are the most suitable and sensitive for use with piscine subjects, this study and others (Kocan et al., 1982; Hose et al., 1982,1983) found that the efficiency and sensitivity of AA analysis makes it preferable. The sampling of resident populations has been performed with embryo analysis which has *further provided an indication of the possible sensitivity of embryo's and this approach (Longwell and Hughes, 1980).*

The use of embryo's should conceivably make the analysis of AA's more sensitive than with adult fish, because of the inherent sensitivity of the embryo and the rapid mitotic division providing a large number of anaphases for analysis. A comparison can be made for English sole exposed to Benzo-a-pyrene via two approaches. Juvenile sole (30 gm.) injected intraperitoneally with 0.05 ug/g (50 ppb) had a significantly increased SCE level at day 7 after injections on days 0 and 3 (Stromberg et al., 1981) while sole embryo's exposed to 2.1 ug/L (ppb) had a significantly higher AA rate than control as gastrula's (Hose et al., 1983). While the exposure methods are dissimilar the sensitivity of the embryo AA analysis is apparent. In a study with sea urchins, Hose et al. (1982) found additionally that AA's were significantly higher in chemical concentrations well below those causing teratogenesis. The speed and ease of analysis of AA's and perhaps micronuclei make this approach the most sensible and cost/time efficient.

Some of the types of AA's seen in this study are presumed to be cyto-toxic (lethal to the cell) to the immediate daughter cells of the dividing parent (Longwell, 1978). Two of the 4 major types of damage found in this study (attached and acentric fragments) are consistent with the alkylating properties of EMS, causing breaks in the DNA sequence. The gaps and breaks observed by other researchers at metaphase result in acentric and attached fragments at anaphase (Nichols et al., 1977), eventually resulting in micronuclei (Schmid, 1975; Kocan et al.,1982). Single bridges, lagging chromosomes and multipolar spindles result in abnormal numerical distribution of chromosomes. It has been stated (Longwell, 1978) that multiple bridges are a toxic phenomenon, however their induction by EMS raises question's about this. The finding that multiple bridges are accompanied by damage regarded as truly genotoxic (acentric and attached fragments) appears to indicate that they really are cytogenetic in origin. Any event potentially affecting DNA distribution, structure or functional capability must be regarded as mutagenic in character, regardless of whether such damage is direct (interaction with DNA) or indirect (interaction with proteins of either the replication or repair mechanism).

The significance of the genotoxic (mutagenic) damage seen in dividing embryo cells includes the immediate death of the cells involved, but is indicative of more subtle heritable changes or the potential for their induction. The embryo system discussed here-in provides a unique opportunity to assess the consequences of mutagenic damage. The potential ability to assess the mutagenic, carcinogenic and toxic *impact of chemicals in the same system, with full siblings for al 1 types of study is unrivalled in toxicology, mutagenesis, and carcinogenisis reseach. The efficiency of embryo's for assessing mutagenic/genotoxic damage due to EMS leads to the speculation that this system, which has been used in field investigations (Longwell, 1978; Longwell and Hughes, 1980) and laboratory investigations (this study;Hose et al., 1982,1983) is equally applicable to in-situ exposures and will provide a very sensitive measure of the genotoxic/mutagenic potential of waterborne chemicals.*

SUMMARY

1) Brachydanio rerio proved to be a very suitable test species, being capable of consistently producing a large number of embryo's for studies with minor variability.
2) A growth reduction was induced by EMS at sub-lethal concentrations, the gastrula and mid-embryo being the most sensitive periods, based on developmental stage, while growth rate (mitotic index) showed an insignificant reduction at 12 and 24 hours with increasing dose.
3) The analysis of micronuclei indicated an insignificant increase in genetic damage at 24 hours of age in an extra-embryonic tissue.
4) The total frequency of anaphase aberrations proved to be a very sensitive indicator of genetic damage due to EMS. Comparison with published reports indicates that this approach is as sensitive as any yet reported in fish, with numerous advantages over other approaches.
5) The relative sensitivity of the endpoints monitored in this study are as follows:

   Anaphase Aberrations > Micronuclei > Mitotic Index > Developmental Stage > Survival

CONCLUSION

The use of anaphase aberrations in Brachydanio rerio embryo's provides a very sensitive measure of aquatic chemical genotoxicity.

RECOMMENDATIONS

1) Validation of this test approach should continue with other chemical genotoxicants and toxicants to determine it's reliability for detecting a variety of classes of compounds. Further investigation of the micronucleus assay in embryo's is warranted.
2) An investigation of the relationship between the genotoxic damage seen in embryo's and eventual health effects in older fish should be instigated, including reproductive effects, mutagenesis, teratogenesis and carcinogenesis.

ACKNOWLEDGMENTS

The author's wish to thank the Ontario Ministry of the Environment for their support of this project, and the supervisory committee for I.R.S. composed of Drs. J.B. Sprague, H.F. Ferguson, D.R. Rokosh and Mr. G. R. Craig for their aid and encouragement. The help, technical expertise, enthusiasm and space provided by the following persons and groups is gratefully acknowledged: Mr. T. Lovasz, Dr. M. Goldberg, and the Biohazards Laboratory and Toxicity Unit's of the Ministry of the Environment. A special thanks is extended to D. R. Walker for graphical and technical assisstance as well as for critically reviewing this manuscript.

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THE EVALUATION AND APPLICATION OF
PULSED NUCLEAR MAGNETIC
RESONANCE
IN THE
ANALYSIS
OF ENVIRONMENTAL SAMPLES

A. King and J.W. Easton
Department of Chemical,
Biological, and Material
Technology,
Ryerson Polytechnical Institute

ABSTRACT

THE EVALUATION AND APPLICATION OF PULSED NUCLEAR MAGNETIC RESONANCE IN THE ANALYSIS OF ENVIROMENTAL SAMPLES

The possibility of applying the low resolution pulsed N M R technique to the analysis of enviromental samples is described. The transversal magnetization for solids decays much faster than that of liquids. Comparison of the magnitudes of the total magnetization measured at different times can be used to determine the solid/liquid ratio or the percentage of liquid in a sample. The theoretical basis will be described along with the assessment of some of the microprocessor programs presently available for the analysis for appropriate samples.

1. INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy has, for the past twenty-five years, been on important instrument in the analysis of molecular systems. The literature abounds with references to the application of this technique to such diverse problems as the elucidation of molecular structure, stereochemical assignments, and the study of dynamic systems.

The past fifteen years has seen a dramatic change in the technology of NMR instrumentation and with it has come an even greater diversity of NMR applications. Pulse Fourier transform (PFT) high resolution spectrometers, some with magnetic field strengths approaching 150,000 gauss and interfaced with a high speed computer, have brought the detection of many magnetic isotopes to levels that make their spectra almost as routine as those of ^{1}H. These instrumental improvements have also facilitated the measurement of molecular relaxation times thereby adding another dimension to the understanding of molecular characteristics (1-3).

In the last ten years, a somewhat different technological approach has emerged. Low resolution pulsed NMR has been applied to a number of diverse chemical situations particularly of a quantitative nature. This method has been used, for example, to determine moisture in food products (4), the ratio of bound-to-free water (5), the fat content and solid-to-liquid ratio of chocolate (6), and the oil in peanut seeds (7). These applications-----fast, reliable, accurate, and a minimum of sample preparation-----suggested a potential for pulse NMR in environmental samples, particularly in the analysis of soils for moisture and organic levels and PCB's in lubricating oils.

Therefore, in conjunction with the Ministry of the Environment Laboratories, the following objectives were set out:

1. to assess the application of low resolution pulsed NMR to particular enviromental samples.

2. to derive suitable experimental metholds in terms of:
    - reproducibility
    - reliability
    - ready calibration
    - speed of analysis
    - ease of operation

2. THE NMR EXPERIMENT

When a molecule bearing hydrogen atoms is placed into a magnetic field B_0, the magnetic moments of the ^1H nuclei precess about the direction of B_0 (along the z-axis) at a rate given by their Larmor frequency (Figure 1)

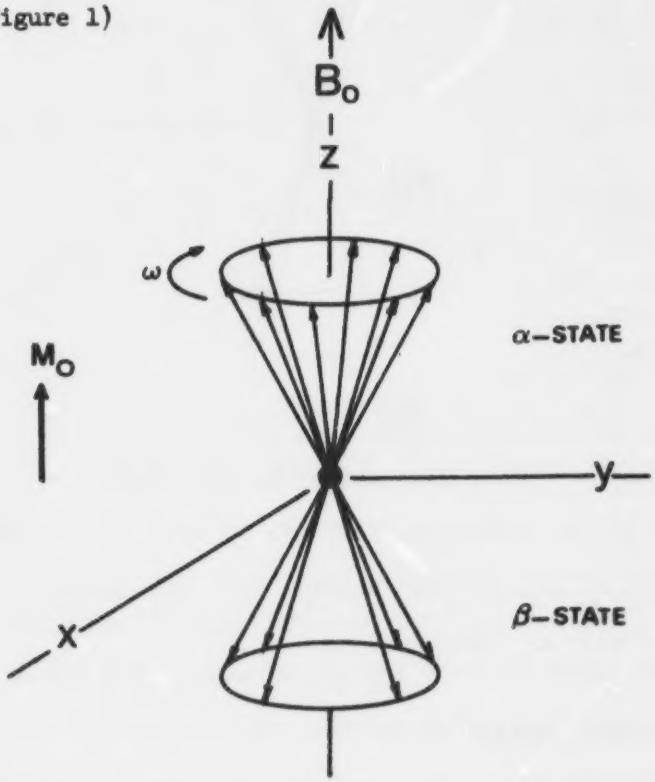

FIGURE 1. The Nucleus in a Magnetic Field

When an observing frequency B_1, applied along the x-axis and polarized in the xy-plane, exactly matches the Larmor frequency of a proton (or group of protons) in the molecule, then a small resultant magnetization in the xy-plane generates a signal (voltage) in the y-axis. (Figure 2)

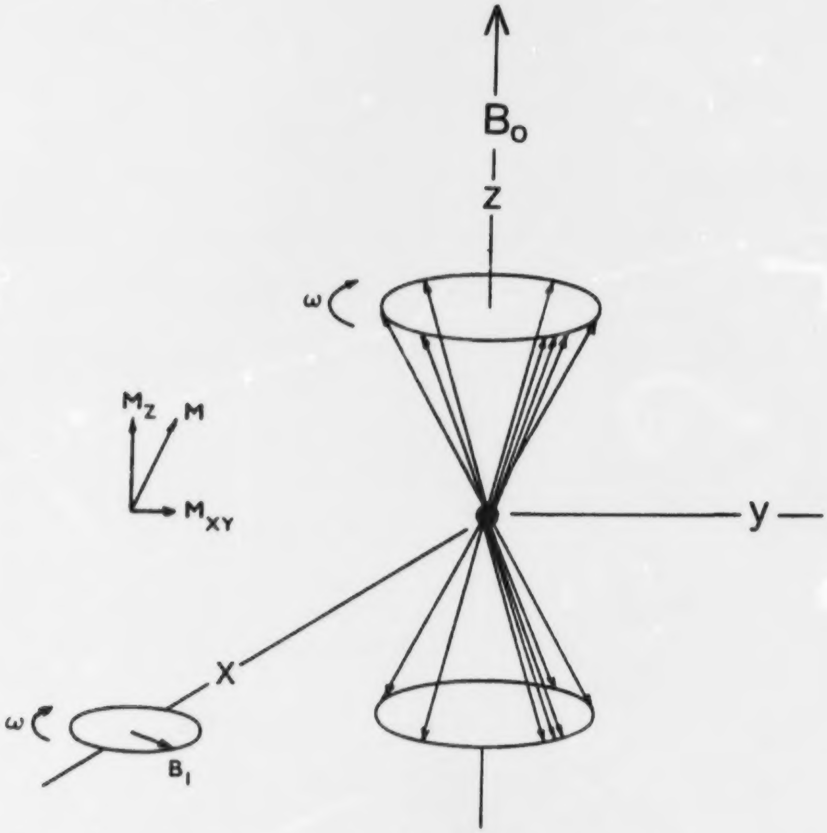

FIGURE 2. Signal Generation in the NMR Experiment

However, if the observing frequency is applied as a short (usec) burst or pulse of high rf (225 watts), then all protons in the sample are simultaneously excited.

3. THE PULSE NMR EXPERIMENT

The pulse nmr experiment is simplified by considering the nuclei in a rotating frame of references. In the rotating frame of reference. In the rotating frame, the entire coordinate system rotates at the Larmor frequency corresponding to the external magnetic field B_o. The rd field B_o, also oscillating at the Larmor frequency, is therefore fixed in the rotating frame.

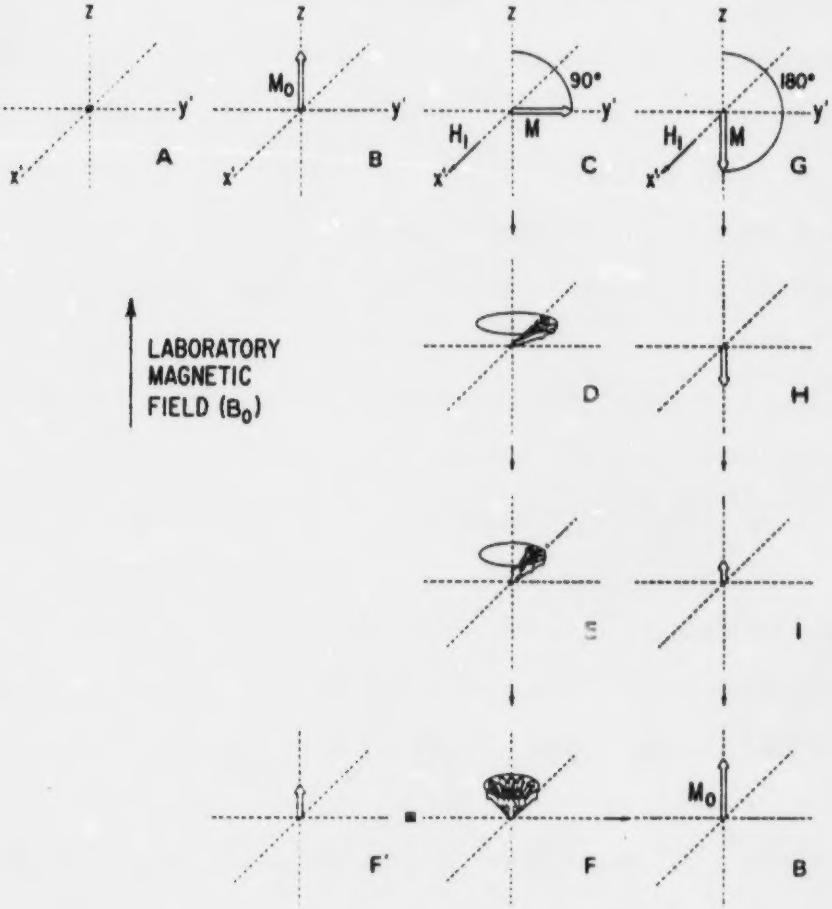

FIGURE 3. The Pulse NMR Experiment in the Rotating Frame

NMR excitation and relaxation in the rotating frame are depicted in Figure 3. The sum of all the individual nuclear magnetic moments is represented by M. When the sample is first introduced into the magnetic field, B_o, no polarization of the nuclear spins is present.

However, the equilibrium magnetization M_0(3B) is quickly established by the interaction of the proton nuclei and the lattice thereby giving an excess of nuclei in the lower energy state according to the Boltzmann distribution law.

When the sample is irradiated by an rf field along the x-axis in the rotating frame, the sample magnetization M is tipped out of alignment with B_0 (the z-axis) and toward the y-axis. The length of pulse determines the angle through which M is tipped; an experimentally determined time (usually 1-100 μsec) will tip M by 90° (3C) whereas twice the pulse width will completely invert M (3G).

Immediately following every excitation pulse, the process of relaxation begins whereby the nuclear system loses its absorbed energy and returns to its equilibrium magnetization M_0. There are two relaxation processes that can occur simultaneously:

1. spin-lattice relaxation (along the z-axis)
2. spin-spin relaxation (in the xy-plane)

Figures 3H and 3I show the return of M to M_0 along the z-axis following a 180° pulse. The rate of return is a first-order process having a rate constant equal to $1/T_1$ where T_1 is called the spin-lattice relaxation time.

Following a 90° pulse, where M coincides with the y-axis, the signal decays both the z-axis and the xy-axis and in the xy-plane. The latter decay phenomenon, as a function of time, is the free induction decay (FID) from which a normal frequency-domain spectrum can be derived. The decay of the xy magnetization is essentially a dephasing of the magnetic moments and the decay rate is first-order with a rate constant of T_2, the spin-spin relaxation time.

Free induction decay signals decay normally as a function of T_2^* where the latter relaxation time includes contribution to dephasing from the inhomogeneity of the magnetic field.

4. THE PULSED NMR INSTRUMENT AND OPERATION

The Bruker Minispec PC 20 is pulsed NMR instrument having a permanent magnet of 4700 gauss and operating at a frequency of 20 MHz. A built-in microcomputer and programmable pulse generator allow any pulse sequence to be entered and used. The microcomputer controls all the data handling and calculations as well as monitoring the magnet temperature and homogeneity, the power supplies, and the signal amplitudes. In addition, the microcomputer is capable of signal averaging which improves the signal-to-noise (S/N) and therefore the accuracy of results.

This technique seems to be well suited to analysis of environmental samples most particularly in the solids content determinations.

a) SOLID/LIQUID RATIOS

Solid-to-liquid ratios are determined based on the rapid decay of magnetization of solids relative to that of liquids. When a 90° pulse is applied to the sample, the signal begins to decay (the free induction decay). However, due to the dead time of the receiver (Figure 4), it is not possible to measure the initial signal magnitude $(s+1)$, but only $(s'+1)$ which is the signal magnitude a certain time after the 90° pulse. However, a correction factor f can be determined experimentally so that the direct ratio of solid-to-liquid can be calculated (8)

$$\%\ \text{solid} = \frac{fs' \times 100}{fs' + 1}$$

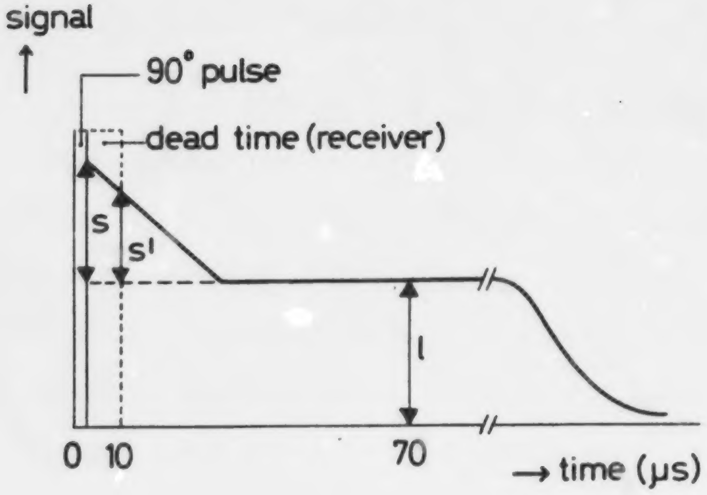

FIGURE 4. Magnetization Decay of a Solid/Liquid Sample

This operation is fully automatic and is begun as soon as the sample tube is inserted into the instrument. The signal is

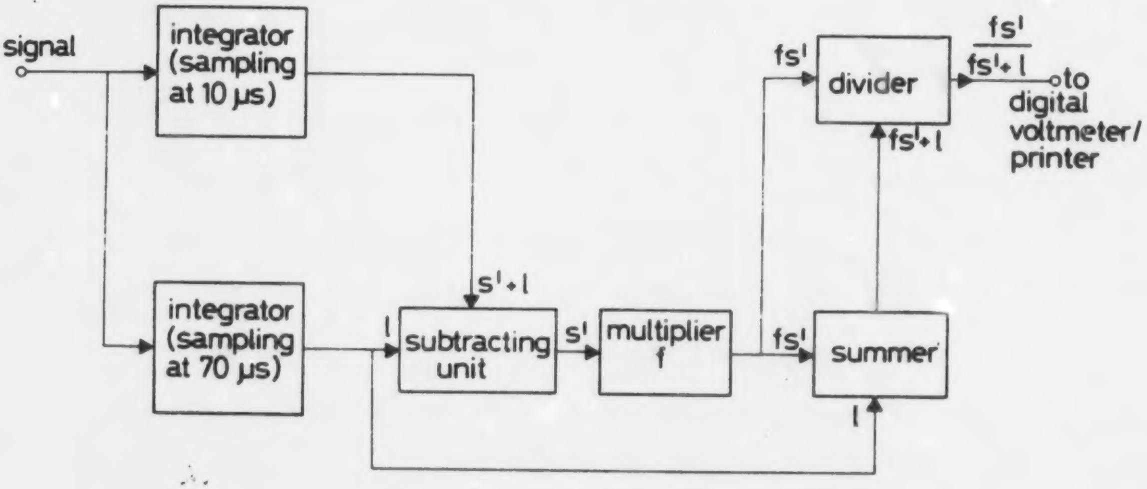
signal
integrator (sampling at 10 μs)
integrator (sampling at 70 μs)
s'+l
l
subtracting unit
s'
multiplier f
fs'
summer
l
fs'
divider
fs'+l
fs'/(fs'+l)
to digital voltmeter/ printer

FIGURE 5. Data Processing Operation

sampled at 10 μs and at 70 μs and the respective amplitudes fed into the data processing unit (Figure 5) of the microcomputer (9). The calculated S/L ratio is then printed out and displayed on a digital voltmeter.

b) PROTON QUANTITIES IN SOLIDS OR LIQUIDS

By shifting the point of signal measurement, the proton quantities can be determined. As shown in Figure 4, signal measurement at 70 μs will be due only to the liquid component. A calibration curve (Figure 6) can be constructed by using standard samples. All the information, including a least squares fit of the data, is stored in the microcomputer so that when a sample of unknown concentration is inserted into the instrument, the concentration is rapidly determined are printed out.

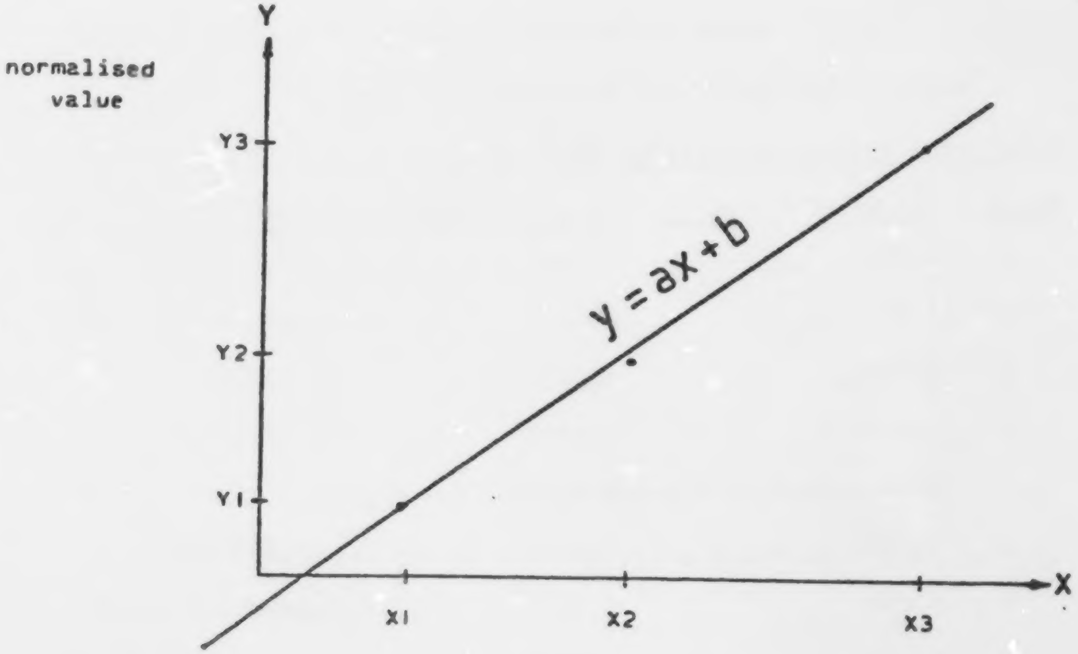

FIGURE 6. Normalization Calibration Curve

5. EXPERIMENTAL AND RESULTS

The Bruker PC 20 Multispec NMR was delivered in May 1983. Initial objectives were as follows:

- (i) to learn the operation of the instrument and to determine methods of calibration.
- (ii) to analyze samples of known concentration in the assessment of various microcomputer programs that are available.
- (iii) to determine the instrument parameters (number of scans, delay time, attenuation, initial delays, etc.) appropriate for a given analysis.

a) CALIBRATION

Standard samples of water in silica were prepared and analyzed.

Three methods of sample preparation were utilized:

1. individual samples having a known % H_2O
2. standard addition method from a single individual sample
3. sealed glass tubes containing known weights of H_2O.

A sample of the results is shown in Table One.

TABLE ONE. Typical Calibration Data for Water in Silica

Sample	Weight of Silica (g)	Weight of H_2O (g)	% H_2O
1	2.9887	0.0307	1.01
2	3.1042	0.0646	2.04
3	2.9448	0.1090	3.57
4	3.2275	0.1617	4.77
	phase-sensitive detector correlation 0.997		
5	3.2330	0.5212	13.88
6	3.2671	0.6585	16.86
7	3.4361	0.7557	18.03
8	3.3421	1.0917	24.62
	diode detector correlation 0.999		

While all three preparation methods gave essentially the same results, it was evident that the use of sealed calibration tubes negated any interactions likely to occur in natural samples. It was concluded that the more conventional sample preparation methods would be used in subsequent work. In addition there was no appreciable difference in these results when the 90° pulse or the 90°-τ-180° pulse sequence were used.

A typical moisture analysis is recorded in Table Two. Although instrument calibration may take 15-30 minutes, the analysis of an individual unknown is complete in only a few minutes.

TABLE TWO. Analysis for Moisture in Sand

Sample	Mass of Sand (g)	Mass of H_2O (g)	% H_2O
1	2.0157	0.0000	0.00
2	2.0129	0.0995	4.71
3	2.0160	0.1994	9.00
4	2.0194	0.2977	12.85
5	2.0130	0.3957	16.43

correlation	0.993
RD	1.2 S
BW	high
mode	PSD
ATT	18
ENH	9

A sample having 8.60% H_2O in sand was analyzed four times and the average value was 8.84%.

b) SOIL/WATER

Soil samples were dried at 105°C and calibration samples prepared. Attempts to calibrate the instrument proved futile since the presence of paramagnetic materials in the roil reduced considerably the relaxation time of the water.

Recent experiments are focussing on the spin-echo pulse sequence (90°-τ-180°) which should eliminate this limitation.

ACKNOWLEDGEMENTS

The authors are grateful to the Ontario Ministry of the Environment for financial supports.

In particular, our appreciation is extended to Dr. M. Moselhy and Dr. O. Meresz for their help and useful discussions.

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Aquatic Toxicity Studies of Multiple Organic Compounds

The Concentration Addition Model

by

George Ozburn[1] and Alasdair Smith
Department of Biology
and
Donald Orr, Charles Mallard and Ainsley Bharath
Department of Chemistry

Aquatic Toxicity Research Group
Lakehead University
Thunder Bay, Ontario
P7B 5E1

[1] Present Address: Technology Transfer Conference No. 4.
Ontario Ministry of the Environment
Toronto, November, 1983

Abstract

A discussion of the various models proposed by aquatic toxicologists for predicting the joint toxicity of mixtures of organic compounds from their individual toxicities is presented. The rationale for adopting the "concentration addition" model as the most relevent to acute toxicity in fish is demonstrated by mixture data generated by our research group on chlorinated benzenes and phenols.

Whether or not concentration addition can successfully predict chronic effects such as fish growth presently remains uncertain because of an inadequate data base for chlorinated benzenes and phenols.

Bioconcentration data on chlorinated benzene mixtures clearly demonstrates that this group of organics bioconcentrate in fish independently of each other, which is consistent with concentration additivity. However, independent bioconcentration is only successful as a predictive model when BCFs are compared on a lipid weight basis.

Introduction

Water quality criteria have traditionally been based on no-effect levels of single toxicants. But the real aquatic environment experiences a continuous bombardment of many toxic substances. Effluents from industrial operations and sewage treatment plants (STPs), along with atmospheric fallout, always contain several pollutants of an organic and/or inorganic nature. Many of these toxicants, particularly the organics referred to as "priority pollutants" (Keith and Telliard, 1979) have been studied extensively in terms of their individual toxicity to aquatic organisms and their potential to concentrate in biological systems. Surprisingly, the joint action of mixtures of organic toxicants is a research area which has received limited attention from aquatic toxicologists. In addition, those responsible for establishing water quality standards have rarely addressed the real issue of multiple organic contamination of aquatic ecosystems. Therefore, the purpose of the investigation by the Aquatic Toxicity Research Group at Lakehead University is to experimentally validate the most appropriate model (or models) proposed by aquatic toxicologists for predicting the joint toxicity of organic mixtures at both the acute and chronic levels. A further objective is to determine whether or not any of the proposed models are useful in predicting bioconcentration of multiple organic compounds.

A brief discussion of the types of joint action arising from mixtures of toxicants is necessary to appreciate the method of approach taken in this study.

A classification by Bliss (1939), which was later expanded by Plackett and Hewlett (1952), describes four types of joint action: 1) simple similar; 2) independent; 3) complex similar; and, 4) dependent (Fig. 1).

A toxicant causes a response by producing changes in a physiological system referred to as the site of action. Similar joint action occurs when two toxicants act at a common site. Conversely, dissimilar joint action arises when the toxicants act at different sites. Such joint action can be interactive if one toxicant influences the biological action of the other or, non-interactive if neither toxicant influences the effect of the other. Mathematical expressions for quantifying joint actions with this system of classification can be readily developed for binary mixtures. However, when more than two chemicals are present, joint action of different pairs can fall into different classes because additional joint actions are possible between the pairs. Therefore, a quantitative description for mixtures of more than two components is not possible when interaction is present; that is, when the joint action is complex similar or dependent.

In a recent publication by Konemann (1981), a Mixture Toxicity Index (MTI) was proposed for comparing quantitatively the results of acute multiple component toxicity tests (Fig. 2). The main advantage of Konemann's mixture toxicity scale over other mathematical expressions such as the Additivity Index proposed by Marking (1977) is that constant values are obtained for two reference points, "concentration addition" and "no addition", independently of the number of compounds and the ratio between the concentrations. These reference points require

Fig. 01. Classification of joint toxic actions (modified from Plackett and Hewlett, 1952).

	Similar joint action	Dissimilar joint action
Non-interaction	simple similar	independent
Interaction	complex similar	dependent

Fig. 02. The Mixture Toxicity Index (MTI) - a mixture toxicity scale (modified from Konemann, 1981).

MTI	Classification of possible types of joint action
MTI < 0	Antagonism
MTI = 0	No addition (independent, r = +1)
0 < MTI < 1	Partial addition
MTI = 1	Concentration addition (simple similar)
MTI > 1	Supra-addition (potentiation or synergism)

further discussion.

The mathematical model for simple similar action is the "toxic unit" concept (Sprague, 1970). Each component of a mixture can be substituted by an equitoxic concentration of an other chemical with simple similar action without altering the reponse. This procedure is called concentration addition by Anderson and Weber (1975).

The no addition reference point is the case of independent action. It occurs when the components of a mixture affect different sites of action but the ultimate reponse is the same - mortality. Anderson and Weber refer to the procedure for calculating the response to this type of joint action as response addition. Independent action can be readily calculated when there is a positive correlation ($r = +1$) between susceptabilities of the individual test organisms to the components of the mixture. This relationship, called the "tolerance correlation" by Konemann, cannot be negative for multiple component mixtures and response addition with $r = 0$ (no tolerance correlation) cannot be used to predict the toxicity of such mixtures with reasonable accuracy (Konemann, 1981).

The MTI, therefore, offers us two mathematical models which have fixed values and for which toxicity experiments can be designed to quantitatively evaluate the joint toxicity of mixtures.

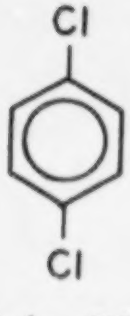
Our approach to the toxicity of multiple organic compounds has been based on the toxic unit concept. The rationale for selecting concentration over response addition as the most useful model for predicting joint toxicity was supported by two criteria:1) historically, pharmacologists and other scientists believe that independent action (response addition) is less likely to occur in reality over other forms of joint toxicity because an organism is at least largely a coordinated whole (Plackett and Hewlett, 1967; Konemann, 1981); and, 2) the nature of the chemicals selected for study - the chlorinated benzenes (CBs) and the chlorinated phenols (CPs). The second criterium is perhaps the most important.

The CBs and CPs which we are studying are illustrated in Figs. 3 and 4. They are first and foremost organic compounds and, despite their structural differences, they do have a common denominator. They are all derivatives of the benzene ring structure and therefore, are more or less lipophilic chemicals. Lipophilic (or hydrophobic) compounds can have a specific mode of toxic action (i.e. primary site of action). For example, there is evidence that PCP interferes with mitochondrial oxidative phosphorylation (Weinbach and Garbus, 1965). However, lipophilic compounds also induce narcotic effects in mammals and fish - membrane perturbation, leading to anesthesia and progressive central nervous system depressions - which are chemically nonspecific (Hermens and Leeuwangh, 1982; Veith *et al.*, 1983). The CBs are excellent examples of narcotic chemicals with a secondary (nonspecific) site of action. But every lipophilic compound can exert this narcotic effect even though it may not be apparent with chemicals like PCP due to the overwhelming effect at the primary (specific) site of action.

We think the joint toxicity of CB and CP mixtures is best

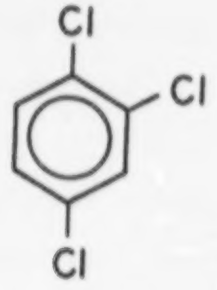
Fig. 03. Chlorinated benzene compounds studied.

1,4 -dichlorobenzene



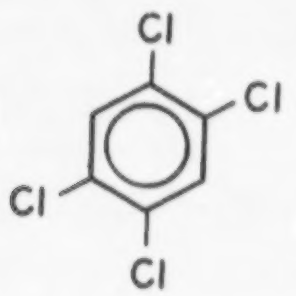
1,4 - DCB
m.w. = 147.01
m.p. = 53 C

1,2,4 - trichlorobenzene



1,2,4 - TCB
m.w. = 181.46
m.p. = 17 C

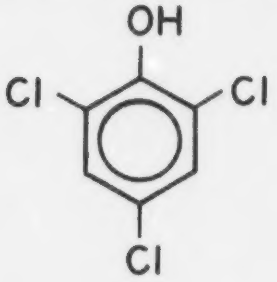
1,2,4,5 - tetrachlorobenzene



1,2,4,5 - TTCB
m.w. = 215.90
m.p. = 139 C

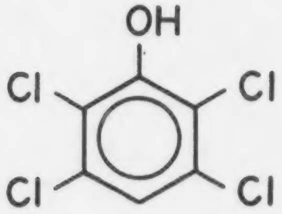
Fig. 04. Chlorinated phenol compounds studied.

2,4,6 - trichlorophenol



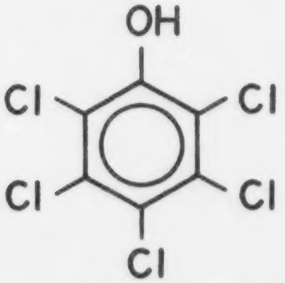
2,4,6 - TCP
m.w. = 197.46
m.p. = 68 C

2,3,5,6,- tetrachlorophenol


OH
Cl
Cl
Cl
Cl

2,3,5,6 - TTCP
m.w. = 231.91
m.p. = 115 C

pentachlorophenol


OH
Cl
Cl
Cl
Cl
Cl

PCP
m.w. = 266.35
m.p. = 191 C

characterized by simple similar action due to the narcotic effect exhibited by these compounds. The most relevent model, therefore, is Konemann's equation for concentration addition (Fig. 5). To be sure, interaction would result in complex similar and/or dependent joint actions, but we have no mathematical model to quantitatively predict these types of joint toxicity.

So far, we have only discussed joint toxicity in terms of acute response (i.e. mortality). Is concentration addition for CB and CP mixtures a relevent model for predicting chronic effects such as fish growth or is response addition more appropriate for sublethal parameters? Very little information is available in the world's literature on chronic joint toxicity. Alabaster (1981) has stated that the few chronic studies with mixtures of toxicants that have been carried out indicate a less-than-additive effect on fish growth and reproduction. However, there is no evidence that chronic responses are non-additive (i.e. independent). A logical approach, therefore, was to extend the toxic unit concept into the design of our Early Life Stage (ELS) toxicity tests to confirm Alabaster's report that chronic joint toxicity demonstrates partial addition.

To be able to predict the bioconcentration dynamics of multiple organic compounds would be extremely useful information. Again, a mathematical expression is necessary in order to evaluate the usefulness of any predictive model for multiple compound bioconcentration.

Our approach towards this aspect of the project was to determine if any of the existing joint toxicity models might be

Fig. 05. The mathematical expression for concentration addition or simple similar action (modified from Konemann, 1981).

$$\sum_{i=1}^{i=n} f_i = 1 \text{ a.t.u.}$$

where: $f_i = \dfrac{\text{water concentration of chemical, } i}{LC_{50} \text{ of } i}$

n = number of components in the mixture

a.t.u. = acute toxic units

Fig. 06. Calculation of the MTI value for equitoxic mixtures (modified from Konemann, 1981; Hermens and Leeuwangh, 1982).

$$MTI = 1 - \frac{\log M}{\log n}$$

where: M = total a.t.u.

n = number of components in the mixture

appropriate for predicting joint bioconcentration. The Structure-Activity Group at the EPA laboratory in Duluth have found in a limited number of tests that organic compounds bioconcentrate in fish independently of each other (Broderius, 1981). The term "independent" in this context, however, should not be interpreted as being synonymous with response addition, discussed under joint toxicity. Non-interactive is perhaps a better descripter than independent in this context. Furthermore, it is well known that organic compounds bioconcentrate in a target tissue - the body lipids. Hence, the lipid tissue can be thought of as a common "site of action". Because interaction is not present, it seems feasible that at least the concept of concentration addition may be appropriate for predicting joint bioconcentration of organic chemicals. Indeed, the mathematical expression for simple similar action , presented in Fig. 5, may even be adaptable for quantifying the process.

With this outline of the objectives and detailed discussion of the approach taken in this study, let us now take a look at the practical application of the concentration addition model for joint toxicity and bioconcentration.

Protocol

Details of the experimental protocol for the toxicity and bioconcentration tests performed during the study was presented last year at this conference (Ozburn et al., 1982). A brief reiteration of the tests may be helpful at this point before reviewing the data generated to date.

1. Acute Testing

Ninety-six hour exposures with flagfish were used to generate LC_{50} values for individual chemicals and for multiple component mixtures. The acute tests employed both static and flow-through techniques. The data reported for the static "rangefinder" tests are based on nominal (non-measured) chemical concentrations, whereas the data generated by flow-through methods utilized measured concentrations determined by gas chromatographic analyses. The LC_{50}s for individual chemicals were calculated using the familiar mg/l (ppm) format. For the mixture tests, mg/l were converted to "acute toxic units" (a.t.u.) according to Konemann's formula for simple similar action (Fig. 5).In order to compare the results of our acute mixture tests, MTI values were also calculated using Konemann's formula for equitoxic mixtures (Fig. 6), and the joint action classified according to his mixture toxicity scale presented in Fig. 2.

2. Chronic Testing

Our ELS toxicity test utilizes two age groups of flagfish in simultaneous flow-through exposures: 1) embryo/larval fish, with data collected on hatching success and 10-day survival; and, 2) one week fry, with data generated on survival and growth (weight) over 28 days.

MATCs are then determined for individual chemicals. The MATC (maximum acceptable toxicant concentration) is defined as the hypothetical toxic threshold concentration falling between the highest concentration showing no effect and the next highest toxicant concentration showing a significant toxic effect (Mount and Stephen, 1967). The hypothetical toxic threshold concentration is called the "chronic value" and is quantified as the geometric mean of the no effect/effect limits. A chronic value is calculated for each response parameter (e.g. hatchability, growth, etc.).

The chronic values determined on individual chemicals are expressed in ug/l (ppb). They are used to convert the concentrations of the mixture's components (also measured in ug/l) to "chronic toxic units" (c.t.u.). by applying essentially the same concentration addition formula as that presented in Fig. 5. In this case, however, f_i is the ratio of the water concentration of component i, to the chronic value of component i. Hence, c.t.u. can be calculated for any chronic response in a multiple component test provided chronic values are obtained for that response in the tests conducted with individual compounds.

3. Bioconcentration Testing

Our flagfish bioconcentration test consists of a 28-day exposure to 5 ug/l of a chemical (the uptake phase) followed by a 14-day depuration period in clean water (the clearance phase). Fish are sampled for total chemical body burden at predetermined intervals during both uptake and clearance.

The bioconcentration data is expressed not only on the basis of total body weight, but also on lipid (weight) - the target tissue or, "site of action".

The only multiple component bioconcentration test that has been conducted so far is a CB mixture. In this presentation we will attempt to apply the principle of concentration addition to the data. We will not attempt, at this time, to develop a quantitative expression for joint bioconcentration until the mixture work with the CPs is completed.

With this brief review of the tests, we can now discuss the data.

Results and Discussion

1. Acute Testing

The non-measured, static tests performed on individual CBs yielded LC_{50} values for flagfish similar to those reported for bluegill under comparable test conditions; the expected trend of increasing toxicity with degree of chlorination is readily apparent in the data (Table 1).

When the flagfish LC_{50} values from Table 1 were used in a triple component CB static to establish a nominal concentration gradient based on Konemann's formula (Fig. 5), an LC_{50} of 1.41 a.t.u. was generated instead of the expected 1.0 value (Table 2). However, a non-measured static is only a "rangefinder" test and has limited precision. Furthermore, the nominal concentration gradient may be too wide to provide a reasonable estimate of the LC_{50} and associated confidence limits, particularly with a data set which lacks partial mortalities (i.e. no per cent dead between 0 and 100).

The single compound, flow-through LC_{50}s generated for 1,4-DCB and 1,2,4-TCB are consistent with the trend of increasing

Table 01. Nominal 96-hour LC_{50}s for 1,4- dichlorobenzene, 1,2,4-trichlorobenzene and 1,2,4,5- tetrachlorobenzene to juvenile flagfish. [a]

CB compound	Trimmed Spearman - Karber Analyses LC_{50}, mg/l flagfish	 bluegill [b]
1,4 - DCB	4.481 (4.022-4.993) [c]	4.28
1,2,4 - TCB	4.000 (3.591-4.454)	3.36
1,2,4,5 - TTCB	2.078 (1.470-2.939)	1.55

[a] non-measured static tests with 24-hour solution renewal and acetone carrier at 196 mg/l.

[b] U.S. EPA, 1978.

[c] 95 % confidence limits.

Table 02. Nominal 96-hour LC_{50} for a triple CB mixture to juvenile flagfish. [a]

Test level	Concentration, [b] acute toxic units	Number exposed	Number dead
0	0	10	0
1	0.25	10	0
2	0.5	10	0
3	1.0	10	0
4	2.0	10	10
5	4.0	10	10

Trimmed Spearman - Karber Analysis

LC_{50}	95 % confidence limits	% trim
1.414	(unreliable)	0

[a] non-measured test with 24-hour solution renewal and acetone carrier at 196 mg/l.

[b] acute toxic units calculated from the concentration addition formula (Fig. 5).

toxicity with degree of chlorination; the apparent anomaly to this trend is 1,2,4,5-TTCB (Table 3). We expected this compound to have the smallest LC_{50}, not the largest.

The TTCB isomer does not fit the expected toxicity pattern because of its poor solubility in water. The solubility of 1,2,4,5 - TTCB at 25 C is ~ 0.5 mg/l (A.T.R.G., 1982).However, concentrations of the chemical measured within the solubility range during the 96-hour acute test produced no mortality (Table 4). Furthermore, follow-up testing with saturated solutions of the compound produced no flagfish mortality after 10 days of exposure, even though toxic signs (narcotic effects) were evident. We concluded that 1,2,4,5 - TTCB is not acutely toxic within 96 hours at water saturation.

But exposure tanks dosed at concentrations above the compound's solubility (test levels 3 to 5) does yield an acute response (Table 4). A detailed discussion of the physico-chemical behaviour of 1,2,4,5 - TTCB was presented at last year's conference (Ozburn et al., 1982) and will not be repeated here. The important point to remember, however, is that the observed toxicity of 1,2,4,5 - TTCB (i.e. the LC_{50} reported in Tables 3 and 4) is a function of both the dissolved portion of the chemical plus the particle size and distribution of the insoluble component.

Using the LC_{50} values from Table 3 in a measured, flow-through CB triple mixture test yields an LC_{50} of 1.4 e.t.u. (Table 5) - the same estimate obtained under static test conditions. It appears that the experimental data does not fit

Table 03. Measured 96-hour LC_{50}s for 1,4- dichlorobenzene, 1,2,4-trichlorobenzene and 1,2,4,5- tetrachlorobenzene to juvenile flagfish. [a]

	Concentration, mg/l		
CB compound	LC_{50}	95 % confidence limits	% trim
1,4 - DCB	2.053	1.955-2.156	0
1,2,4 - TCB	1.217	1.081-1.371	0
1,2,4,5, - TTCB	2.150	1.853-2.495	35

[a] flow-through tests with acetone carrier at 196 mg/l.

Table 04. Acute toxicity of 1,2,4,5 - tetrachlorobenzene to juvenile flagfish. [a]

Test level	Concentration, [b] mg/l	Number exposed	Number dead
0	0	20	0
1	0.148±0.009 [c]	20	0
2	0.373±0.055	20	0
3	0.695±0.050	20	4
4	1.855±0.326	20	7
5	2.493±0.357	20	13

Trimmed Spearman - Karber Analysis

LC_{50}	95 % confidence limits	% trim
2.150	1.853-2.495	35

[a] flow-through 96-hour test with acetone carrier at 196 mg/l.

[b] glass sintered samples.

[c] mean ± s.d.

Table 05. Acute toxicity of a triple CB mixture to juvenile flagfish. [a]

	Concentration, acute toxic units [b]					
Test level	1,4-DCB a.t.u.	1,2,4-TCB a.t.u.	1,2,4,5-TTCB a.t.u.	Total a.t.u.	Number exposed	Number dead
0	0	0	0	0	40	0
1	0.211	0.220	0.172	0.604	40	0
2	0.194	0.237	0.224	0.655	30	1
3	0.230	0.276	0.255	0.761	10	0
4	0.457	0.390	0.159	1.006	10	3
5	0.371	0.440	0.382	1.193	30	1
6	0.434	0.502	0.413	1.348	10	5
7	0.733	0.683	0.112	1.528	10	10
8	0.501	0.583	0.609	1.693	10	2
9	0.882	0.814	0.155	1.851	10	10

Trimmed Spearman - Karber Analysis

LC_{50}	95 % confidence limits	% trim	MTI value
1.402	1.296 - 1.517	0	0.69

[a] flow-through 96-hour test with acetone carrier at 196 mg/l.

[b] acute toxic units calculated from the concentration addition formula (Fig. 5).

the concentration addition model. Applying Konemann's equation from Fig. 6, the calculated MTI value is 0.69 and the joint action would be classified as partial addition: 0< MTI < 1 (Fig. 2).

In experiments with guppies, Konemann (1981) also found that a mixture of DCB, TCB and TTCB generated an LC_{50} estimate of 1.4 a.t.u. when the mixture's toxic strength was calculated from experimental LC_{50}s determined on individual components. But when he calculated the individual LC_{50}s on the basis of their QSARs (Quantitative Structure Activity Relationships) he discovered that the mixture's toxic strength was exactly equal to 1 a.t.u. and hence, fit the concentration addition model for simple similar action. His explanation for the discrepancy in the experimental data was that the CBs do not have the same primary site of action. They do have at least one common site of action which is represented by the QSAR calculations, but that common site of action is primary for some CBs and secondary for others.

Our explanation as to why the experimental data does not fit the proposed model is based on TTCB's lack of acute toxicity at water solubility.

The relative contribution of 1,2,4,5 - TTCB to total toxicity at every test level was very small compared to the contributions of the DCB and TCB components. In fact, at test level 8 where the TTCB component was at its maximum contribution to the total a.t.u. value, an actual decrease in mortality was observed (Table 5). We think TTCB was contributing little or nothing to the mixture's total toxic strength.

If we remove the TTCB component and reanalyze the triple CB mixture data using only the DCB and TCB components to calculate the concentration gradient, we generate an LC_{50} of 1.07 a.t.u.; the equivalent MTI value is 0.90 (Table 6). The same data set utilizing the upper 95 % confidence limit of the individual LC_{50}s for 1,4, - DCB and 1,2,4 - TCB (from Table 3) will yield an LC_{50} value for the test of 0.99 a.t.u.

It appears that DCB and TCB do exhibit simple similar action while TTCB does not. TTCB may or may not have the same common site of action as suggested by Konemann, but we know that it is non- toxic at water solubility (within 10 days) and therefore concentration addition is not demonstrated when the compound's LC_{50} is based primarily on suspended particles of unknown toxicity and unknown mode of action.

We did attempt to follow-up the CB triple mixture test with a flow-through DCB/TCB binary test. However, analytical problems occurred with the water analyses during the actual test, which were not discovered until after the test was completed. Hence, the only reliable water chemistry available was a single preliminary analysis performed after the flow-through system was stabilized but two days prior to introducing the test fish. Although this test cannot be regarded as a valid demonstration of DCB/TCB concentration addition, we did take the trouble to "crunch the numbers". Using the upper 95 % confidence limit of the LC_{50} estimates for 1,4 - DCB and 1,2,4 - TCB from Table 3, the biological response, when fitted to the single chemistry analysis, yields a 95 % confidence limit for the LC_{50} of 1.024 to 1.312 a.t.u. The MTI value for 1.024 a.t.u. is 0.97.

Table 06. Acute toxicity of a triple CB mixture WITHOUT the TTCB component to juvenile flagfish. [a]

	Concentration, acute toxic units [b]				
Test level	Total a.t.u.	- 1,2,4,5-TTCB a.t.u.	= (1,4-DCB+1,2,4-TCB) a.t.u.	Number exposed	Number dead
0	0	0	0	40	0
1	0.604	0.172	0.432	40	0
2	0.655	0.224	0.431	30	1
3	0.761	0.255	0.506	10	0
4	1.006	0.159	0.847	10	3
5	1.193	0.382	0.811	30	1
6	1.348	0.413	0.935	10	5
7	1.528	0.112	1.416	10	10
8	1.693	0.609	1.084	10	2
9	1.851	0.155	1.696	10	10

Trimmed Spearman - Karber Analysis

LC_{50}	95 % confidence limits	% trim	MTI value
1.074	0.990 - 1.165	1.25	0.90

[a] flow-through 96-hour test with acetone carrier at 196 mg/l.

[b] acute toxic units calculated from the concentration addition formula (Fig. 5).

We think our acute data on CB mixtures supports the concentration addition model of joint toxic action for this group of chemicals.

Results of the non-measured, static tests performed on individual CPs are presented in Table 7. Again, the anticipated trend of increasing toxicity with degree of chlorination is readily demonstrated by the data. Our results, however, are significantly different from those reported for bluegill under static test conditions (Table 7). It's true that non-measured statics do not give particularly accurate LC_{50} estimates, but the large discrepancy between the flagfish and bluegill data cannot be explained solely on the basis of the inadequacies of the static test method. Sensitivity of the species to the toxicant is also not likely to account for these differences.

We think the differences are probably due to two characteristics of the CP compounds: 1) chemical purity; and, 2) effect of water pH on toxicity.

CP compounds can contain varying amounts of impurities, which is usually a result of process chemistry (Jones,1981). The most prominant of these impurities are the polychlorinated dibenzo-p-dioxins , (PCDDs) , and also the polychlorinated dibenzofurans (PCDFs), which are highly toxic compounds in themselves. Commercial grade CPs can vary considerably with repect to the quantities of PCDDs and PCDFs in their composition. Therfore, the chemical purity of a CP compound can result in big differences in reported LC_{50} estimates.

The other important characteristic of CPs is that their

Table 07. Nominal 96-hour LC_{50}s for 2,4,6- trichlorophenol, 2,3,5,6- tetrachlorophenol and pentachlorophenol to juvenile flagfish. [a]

	Trimmed Spearman - Karber Analyses	
	LC_{50}, mg/l	
CP compound	flagfish	bluegill [b]
2,4,6, - TCP	2.264 (2.021-2.537) [c]	0.320
2,3,5,6 -TTCP	0.564 (0.458-0.694)	0.170
PCP	0.186 (0.119-0.292)	0.020-0.060

[a] non-measured static tests with 24-hour solution renewal and acetone carrier at 79 mg/l.

[b] Ontario M.O.E., 1983.

[c] 95 % confidence limits.

toxicity decreases with increasing water pH (Holcombe et al., 1980). The CPs dissociate in water to chlorophenate ions, which are less toxic than the undissociated form. The extent of dissociation increases as pH increases and thus, the toxicity of the compound decreases. Hence, the pH of the diluent water in the flagfish and bluegill static tests reported in Table 7 may account for the observed differences in acute toxicity.

In our study, we have used the purest grades of CPs available, in order to reduce joint effects from highly toxic impurities. As an added precaution, the CPs were purified further in our laboratory by a variety of techniques.

The pH (in the laboratory) of our Lake Superior water supply is ~ 7 (Ozburn et al., 1982).Thus, our near-neutral diluent water does not contribute significantly to dissociation of the CP chemicals under study. The degree of dissociation is almost entirely based on the dissociation constant of the specific CP isomers.

When the flagfish nominal LC_{50} values from Table 7 were used in a triple component CP static test, an LC_{50} of 0.53 a.t.u. was obtained (Table 8). With a result of only half a toxic unit, the data suggests that the supra-addition model would be a better descripter of the joint toxic action of these CP compounds.Indeed, the calculated MTI value for the test is 1.58. However, we proceeded with the concentration addition concept because the single compound, flow-through LC_{50} generated for 2,3,5,6 - TTCP was significantly different from that obtained in the static test. The flow-through LC_{50}s for 2,4,6, - TCP and PCP

Table 08. Nominal 96-hour LC_{50} for a triple CP mixture to juvenile flagfish. [a]

Test level	Concentration, [b] acute toxic units	Number exposed	Number dead
0	0	10	0
1	0.175	10	0
2	0.351	10	0
3	0.702	10	9
4	1.404	10	10
5	2.807	10	10
6	5.614	10	10

Trimmed Spearman -Karber Analysis

LC_{50}	95 % confidence limits	% trim
0.532	0.466-0.607	0

[a] non-measured test with 24-hour solution renewal and acetone carrier at 79 mg/l.

[b] acute toxic units calculated from the concentration addition formula (Fig. 5).

were consistent with the static estimates but 2,3,5,6 - TTCP gave an LC_{50} estimate two-fold larger (Table 9). Now we needed an explanation as to why the static and flow-through techniques yielded such different results for the TTCP compound.

Although both test methods gave numbers which were consistent with the trend of increasing toxicity with increasing chlorination, the flow-through LC_{50} of 1.16 mg/l for TTCP appeared to be a more reasonable estimate of its toxicity in relation to TCP and PCP. Besides, we tend to accept a measured flow-through estimate as a more valid number.

Because we decided that the static LC_{50} for TTCP was wrong (particularly when the nominal CP mixture test gave a result no where near 1 a.t.u.), we proceeded with a triple CP flow-through test using the LC_{50} estimates from the individual flow-through tests to calculate the a.t.u. This test yielded an LC_{50} value of 0.90 a.t.u., and a MTI value of 1.10 (Table 10). The 95 % confidence limits of the LC_{50} easily brackets 1 a.t.u. and we think the data fits the concentration addition model very nicely.

When we later repeated the 2,3,5,6 - TTCP static test, we got essentially the same LC_{50} as the initial test - 420 mg/l (95 % confidence limits, 316 - 558 mg/l). We can think of only two reasons why 2,3,5,6 - TTCP appears to be twice as toxic under static test conditions.

There is a relatively high loading density (5 juvenile fish per 3 liters of water) in the static jars. Dissolved oxygen levels may be somewhat lower (compared to flow-through

Table 09. Measured 96-hour LC_{50}s for 2,4,6- trichlorophenol, 2,3,5,6- tetrachlorophenol and pentachlorophenol to juvenile flagfish. [a]

CP compound	Concentration, mg/l		% trim
	LC_{50}	95 % confidence limits	
2,4,6 -TCP	2.207	1.995-2.441	0
2,3,5,6 - TTCP	1.164	1.040-1.303	0
PCP	0.218	0.197-0.243	0

[a] flow-through tests with acetone carrier at 79 mg/l.

Table 10. Acute toxicity of a triple CP mixture to juvenile flagfish. [a]

	Concentration, acute toxic units [b]					
Test level	2,4,6-TCP a.t.u.	2,3,5,6-TTCP a.t.u.	PCP a.t.u.	Total a.t.u.	Number exposed	Number dead
0	0	0	0	0	40	0
1	0.043	0.038	0.039	0.119	40	0
2	0.084	0.076	0.080	0.240	40	0
3	0.169	0.153	0.160	0.481	40	0
4	0.337	0.306	0.317	0.960	40	24
5	0.695	0.620	0.640	1.952	40	40
6	1.341	1.212	1.091	3.645	40	40

Trimmed Spearman - Karber Analysis			
LC_{50}	95 % confidence limits	% trim	MTI value
0.899	0.807-1.002	0	1.10

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[a] flow-through 96-hour test with acetone carrier at 79 mg/l.

[b] acute toxic units calculated from the concentration addition formula (Fig. 5).

conditions) and excess CO_2 could favour carbonic acid production, resulting in a depressed water pH. More of the TTCP compound would exist in the more toxic, undissociated form at a lower pH.

Or perhaps, a degradation product (either from metabolism or from physico-chemical breakdown) is being produced which is more toxic than the parent compound.

In any case, the toxic properties of 2,3,5,6 - TTCP under the two test conditions is an interesting phenonomen which we hope to persue at a later date. In the meantime, the next step as far as the acute testing is concerned, is to experimentally test the concentration addition model between the CB and CP classes of compounds. We think our acute data demonstrates that these chemicals are very close to being concentration additive within their own class. It will be interesting to see if the model is also applicable to a CB / CP mixture.

2. Chronic Testing.

In order to test any model for chronic joint toxicity, it is first necessary to bracket the MATC for a specific sublethal response in individual chronic tests performed on a mixture's components. Only when an MATC is bracketed can a chronic value be calculated for that particular parameter. The chronic values provide the database for converting the measured concentrations of the components in a mixture test to c.t.u., in the same way that LC_{50}s on separate components are used to convert the concentrations in a mixture to a.t.u.

A summary of the ELS data generated on individual CB compounds is presented in Table 11. It is readily apparent from this table that we were not always successful in bracketing the MATC and hence, unable to calculate the critical number - the chronic value - for some of the sublethal responses measured in the ELS tests. One of the main problems encountered in establishing effective concentration gradients for individual CBs was the lack of reliable chronic data on these chemicals in the literature. More often than not, we had only our LC_{50} to use as a reference point.

The reported MATC values were, in most cases, boarderline in terms of the biological response being significant or non-significant. A shift of the exposure gradients up one concentration level would probably have resulted in successful bracketing of the no effect / effect intervals. Another problem with the ELS data on individual CB compounds is that there were no chronic tests performed on 1,2,4 - TCB. The TCB data presented in Table 11 is from previous work on dielectric fluids (Smith et al., 1979). This earlier study employed electrical grade TCB which is 66 % 1,2,4 - TCB and 33 % 1,2,3 - TCB.Therefore, the reported chronic values may not be an accurate estimate for pure 1,2,4 - TCB.

Despite these gaps in the chronic database we did proceed with a triple CB, ELS flagfish test selecting the concentration gradients for the compounds on the basis of the data in Table 11. At last year's conference, we presented the biological responses obtained in this test in the form of "interim" tables (Ozburn et al., 1982). We managed to bracket the MATC on 3 out of the 4 sublethal parameters measured, missing only the no effect /

Table 11. Summary of flagfish ELS data collected on individual CB compounds.

	Chronic value and / or MATC (ug/l)		
	1,4-DCB	TCB [a]	1,2,4,5-TTCB
Egg hatchability	MATC >835	chronic value 1432	MATC >238
10-day larval survival	chronic value 252 & 273	chronic value 1133	MATC >238
28-day fry survival	MATC >349	X [b]	chronic value 138
28-day fry growth	MATC >349	X	chronic value 85

[a] electrical grade TCB (data from Smith *et al*., 1979).

[b] no test performed.

effect interval for egg hatchability. However, time has not allowed us to repeat any of the individual tests, and without a complete set of chronic values, we are still unable to convert all of the measured concentrations to c.t.u.

Chronic testing on individual CP compounds is not finished at the present time (Table 12). Again, we already have the problem of an incomplete set of chronic values, even at this stage of the program.

We have also conducted a binary CB mixture test with 1,4 - DCB and 1,2,4 - TCB to evaluate flagfish 10-day larval survival. To demonstrate conversion of the chemical data from ug/l to c.t.u., the results of this test are presented in Table 13, utilizing chronic values of 252 ug/l for 1,4 - DCB and 1133 ug/l for 1,2,4 - TCB. It must be re-emphasized that the 1133 ug/l chronic value is based on electrical grade TCB and may not be an accurate estimate for pure 1,2,4 - TCB.

The CB binary test gave a chronic value of 1.17 c.t.u. (MATC, 1.01 - 1.36 c.t.u.). The corresponding MTI value is 0.77. We think this value is close enough to unity to suggest that concentration addition may in fact be an appropriate model for chronic joint toxicity of CBs. Repeat testing on individual compounds, to complete the chronic database, should be carried out to experimentally confirm the validity of the model.

3. Bioconcentration Testing

If, in fact, organic compounds like CBs bioconcentrate in fish with no interaction present, then we should be able to demonstrate that the parameters used to quantify bioconcentration

Table 12. Summary of flagfish ELS data collected on individual CP compounds.

	Chronic value and / or MATC (ug/l)		
	2,4,6-TCP	2,3,5,6-TTCP	PCP
Egg hatchability	test in progress	MATC >238	chronic value 136
10-day larval survival	test in progress	MATC >238	chronic value 75
28-day fry survival	test in progress	chronic value 136	chronic value 135
28-day fry growth	test in progress	MATC >1037	MATC >179

Table 13. Ten-day survival of flagfish larvae exposed to a binary chlorobenzene mixture.

	Concentration					
Test	1,4 - DCB		1,2,4 - TCB		Total	Per cent
level	ug/l	c.t.u.	ug/l	c.t.u.	c.t.u.	survival
0	0	0	0	0	0	93
1	9.4	0.037	59.5	0.053	0.090	80
2	30.5	0.121	169.5	0.150	0.271	93
3	115.8	0.460	624.5	0.551	1.011	83
4	154.7	0.614	839.7	0.741	1.355	67 *
5	209.7	0.832	1118.0	0.987	1.819	47 **
6	316.0	1.254	1766.0	1.559	2.813	3 **

Lee-Desu Analysis

10-day survival - level 4(*) significant (P < .05)

- levels 5 and 6 (**) significant (P < .01)

Concentration, c.t.u.		
Chronic value	MATC	MTI value
1.17	1.01 - 1.36	0.77

dynamics are approximately equal in magnitude when comparing individual tests to mixture tests. Flagfish bioconcentration data is presently available for individual tests with 1,4 - DCB and 1,2,4,5 - TTCB, as well as a mixture of 1,4 - DCB, 1,2,4 - TCB and 1,2,4,5 - TTCB.

The important bioconcentration parameters are: 1) the uptake rate constant, K_1; 2) the clearance rate constant, K_2; and, the bioconcentration factor, K_B. The experimental values obtained for these parameters in the individual CB tests are presented in Table 14. In order to demonstrate the structure - activity relationship of increasing bioconcentration potential with degree of chlorination, data on 1,2,4 - TCB (extracted from the triple CB mixture test) is also included. The values obtained for the same parameters in the triple CB mixture test are listed below in Table 15.

Both data sets nicely illustrate the structure - activity relationship. But if we compare the K_B values for DCB and TTCB from the individual tests to those obtained for the mixture, it is obvious that the bioconcentration factor for both CBs is considerably larger in the mixture test. This is contrary to what we would expect, assuming that no interaction is present. The K_B values should be approximately the same.

Because lipid is the target tissue or, "site of action" for bioconcentration of organic chemicals, we can take the same data sets from Tables 14 and 15 and express the values for the bioconcentration parameters on the basis of lipid content (Tables 16 and 17). Obviously, the K_B values have been transformed into much larger numbers. They can be compared much more easily, Table 14.Bioconcentration of 1,4 - DCB, 1,2,4 - TCB [a] and 1,2,4,5 - TTCB in juvenile flagfish.

Bioconcentration parameter	mean ± s.d.		
	1,4-DCB	1,2,4-TCB	1,2,4,5-TTCB
Exposure [ ], ug/l	2.68±0.23	3.80±0.23	3.02±0.29
K_1, days^{-1}	291±26	1158±84	1630±246
K_2, days^{-1}	0.98±0.04	0.57±0.01	0.40±0.03
K_B	296±29	2026±154	4050±703

[a] data extracted from triple CB mixture test.

Table 15.Bioconcentration of a triple CB mixture in juvenile flagfish.

Bioconcentration parameter	mean ± s.d.		
	1,4-DCB	1,2,4-TCB	1,2,4,5,-TTCB
Exposure [ ], ug/l	3.28±0.27	3.80±0.23	3.87±0.27
K_1, days^{-1}	565±45	1158±84	1565±107
K_2, days^{-1}	1.32±0.04	0.57±0.01	0.22±0.01
K_B	429±37	2026±154	7048±606

Table 16. Bioconcentration of 1,4 - DCB, 1,2,4 - TCB [a] and 1,2,4,5 - TTCB in lipid of juvenile flagfish. [b]

Bioconcentration parameter	1,4-DCB	1,2,4-TCB	1,2,4,5-TTCB
	mean ± s.d.		
Exposure [ ], ug/l	2.68±0.23	3.80±0.23	3.02±0.29
K_1, days^{-1}	4230±439	10141±775	17100±3185
K_B	3590±498	17750±1481	50300±11427
log K_B	3.56±0.08	4.25±0.04	4.70±0.11

[a] data extracted from triple CB mixture test.

[b] K_B, K_1 lipid = 100/8.5 = 11.8 (K_B, K_1 body weight) and K_2 lipid = K_2 body weight.

Table 17. Bioconcentration of a triple CB mixture in lipid of juvenile flagfish. [a]

Bioconcentration parameter	1,4-DCB	1,2,4-TCB	1,2,4,5-TTCB
	mean ± s.d.		
Exposure [ ], ug/l	3.28±0.27	3.80±0.23	3.87±0.27
K_1, days^{-1}	4947±390	10141±775	13710±944
K_B	3760±318	17750±1481	61740±5232
log K_B	3.58	4.25±0.04	4.79

[a] K_B, K_1 lipid = 100/11.4 = 8.77(K_B,K_1 body weight) and K_2 lipid = K_2 body weight.

however, by coverting them to log numbers.

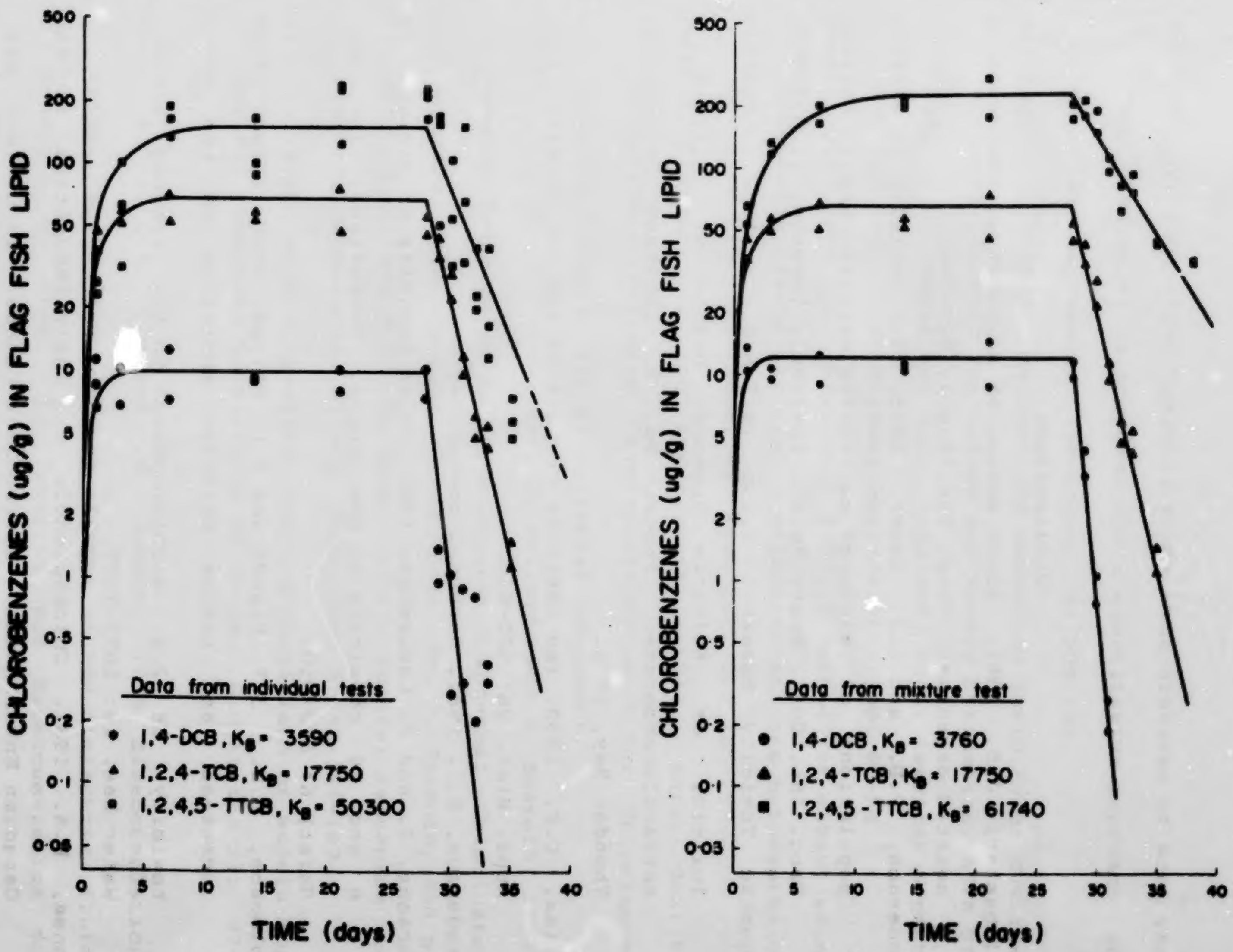
With the data now normalized on the basis of lipid weight, there does appear to be a relationship between individual CB bioconcentration and bioconcentration of CB mixtures. The mean log K_B for 1,4 - DCB and 1,2,4,5 - TTCB from the triple CB mixture can be readily bracketed by the standard deviations of the log K_B value for the same compounds in the individual CB tests. The standard deviations for the log K_Bs for these two components in the mixture test have been intentionally omitted to help clarify this relationship. Within the limits of experimental error, we can conclude that there is no interaction between components of a CB mixture; that is, the CBs bioconcentrate in fish independently of each other. Fig. 7 graphically illustrates both independent bioconcentration and the structure - activity relationship of the CB compounds.

If we also find that no interaction exists in the bioconcentration dynamics of CP compounds, we will attempt to develop a mathematical expression to quantify this relationship. The concentration addition model which has been developed for joint toxicity may very well be an appropriate approach towards quantifying joint bioconcentration.

Acknowledgments

Sam Spivak drafted the figures. This study is jointly supported by the Ontario Ministry of the Environment and by Environment Canada.

Fig. 07. Comparison between CB bioconcentration in individual compound tests and a triple mixture test.



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REMOVAL OF HAZARDOUS CONTAMINANTS (HCs) IN AN ONTARIO WATER POLLUTION CONTROL PLANT (WPCP)

R.J. Rush and L.J. Taylor

SUMMARY

The Ontario Ministry of the Environment (MOE) initiated a study to monitor HCs at an Ontario WPCP in 1982. The objective of the study was to provide an accurate estimate of the concentrations and annual loading of hazardous contaminants entering and being discharged from the WPCP, in order to determine the present sewage treatment process efficiency and factors influencing HCs removal. Parameters monitored included total PCBs, pesticides, PAHs, and trace metals. Conventional parameters were also monitored for reference purposes.

The study is still in progress, and therefore, a summary of the procedures, some analytical QA/QC results and preliminary treatment plant efficiency data will be presented for PAHs only.

ABSTRACT

Until very recently, relatively little was known about the identities and quantities of hazardous contaminants (HC's) entering wastewater treatment plants and little information was available on the factors which would influence their treatability and ultimate fate. Currently in Canada, the data base for HC's occurrence and removal at WPCP's is very limited. Therefore, in August 1982 a study was initiated to provide an accurate estimate of the annual loading of hazardous contaminants entering and being discharged from an Ontario WPCP, in order to determine the present sewage treatment process efficiency and factors influencing HC's removal. Monitoring included selected HC's such as PCBs, pesticides, PAHs, metals and conventional pollution parameters.

The study, which is still in progress, was divided into two phases. In Phase I, sampling was performed at the WPCP on two separate occassions in order to optimize site sampling techniques and analytical methods. Phase 2 involved collecting and analyzing 24 hour composite samples during three separate periods:

1. Winter (December 1982 and January 1983)
2. Spring (April and May 1983)
3. Summer (June to August 1983)

The selected parameters were determined in the raw combined influent and final effluent. The in-plant return was also monitored on certain sampling days. The WPCP operation was observed closely during the sampling periods and any operational upsets were recorded for correlation with the analytical results.

Chemical analyses and evaluation of the data are on-going. A progress report summarizing the study methodology and results available to date will be presented.

INTRODUCTION

Background and Relevance

In the modern industrial society, hazardous contaminants (HCs), both trace metals and organic compounds are discharged into public sewage systems. Until very recently, relatively little was known about the identities and quantities of contaminants entering wastewater treatment plants and little information was available on the factors which would influence their treatability and ultimate fate.

Recently published reports include four dealing with large scale field surveys and two based on studies of pilot-scale wastewater treatment facilities:

1. Survey of 40 Publicly Owned Treatment Works (POTW) by U.S. EPA Effluent Guidelines Division (EPA 1982a).
2. A 30 day study at a POTW by U.S. Effluent Guidelines Division (EPA 1982b).
3. Survey of 25 POTW by U.S. EPA Municipal Environmental Research Laboratory (MERL) (Cohen et al 1981).
4. The 5 plant study by the Chemical Manufacturers Association (CMA) and the U.S. EPA (CMA/EPA 1982).
5. U.S. EPA MERL pilot plant studies of semi-volatile organic compounds (Petrasek et al 1980 and 1981).
6. Pilot plant study of a group of volatile and semi-volatile organic compounds by van Rensburgh et al (1980) of the National Institute for Water Research in South Africa.

Results from the EPA surveys and pilot plant studies indicated that the wastewater treatment processes studied were stable over a wide range of operating conditions and were generally effective in removing toxic substances, as shown in Table 1.

However, the EPA 40 POTW study (EPA 1982a) showed that individual inorganics; e.g. As, Cd, Cu, Hg and Pb, and organics; e.g. polynuclear aromatic hydrocarbons (PAHs) and pesticides pass through a number of treatment plants in amounts and with frequency to be probable cause for concern.

TABLE 1. EXAMPLES OF TOXICS REMOVAL EFFICIENCY IN MUNICIPALS WPCPs

STUDY	REFERENCE	REMOVAL EFFICIENCY
40 POTW Survey	EPA (1982a)	For half of the plants studied: 70% for metals 82% for volatile organics 65% for base-neutral organics
25 POTW Survey	Cohen (1981)	>80% for many organics
Pilot Plant Study	Petrasek (1981)	>90% for the semi-volatiles studied

As influent concentrations of many conventional and priority pollutants increased, effluent concentrations also increased. This implies that the removal rates for the priority pollutants were relatively constant and that a fixed percentage of the loading of these pollutants was removed by secondary treatment.

In general, the higher the industrial contribution to a POTW, the higher the concentration of priority pollutants in the POTW influents. Heavy rainfall increased metallic priority pollutant mass loading at POTWs while the mass loading of both metallic and organic priority pollutants in POTW influents was higher on weekdays than on weekends.

Some pollutants not detected in POTW influents were regularly measured at high levels in the corresponding sludge streams; e.g. PAHs and phthalates which were concentrated to the greatest degree in sludges. In this regard, the survey data [EPA (1982a) and Cohen (1981)] support the findings of Petrasek (1981), who has suggested that sludges, particularly primary sludges are likely to act as a sink for these compounds because of a bioconcentration effect.

In the South African study (van Rensburg et al 1980), van Rensburg reported 90 percent effectiveness in the removal of toxic organics even under the pressure of shock loads of these chemicals, and he observed a severe build-up of some compounds in the recycled sludge. Both of these observations are in agreement with those reported by the U.S. EPA in their surveys and pilot-scale studies (EPA 1982a, Cohen 1981 and Petrasek 1981).

Trace organics monitoring studies at several Canadian sewage treatment plants are in progress (e.g. EPS 1980 and MOE 1980) and continuous flow fate studies of trace organics in a pilot plant at the Wastewater Technology

Centre (WTC) in Burlington, Ontario are on-going. However, currently in Canada the data base for HCs occurrence and removal at WPCPs is very limited (CANVIRO 1983). Therefore, in August 1982 a study was initiated to provide an accurate estimate of the annual loading of HCs entering and being discharged from an Ontario WPCP.

STUDY OBJECTIVES AND SCOPE

Analytical problems have been reported in many toxics studies conducted to date (e.g. EPA 1982a and CMA/EPA 1982), and therefore, the study was divided into two phases.

The specific objectives of Phase 1 were:

1. To identify the most significant HCs in the WPCP influent, effluent and sludge streams.
2. To establish a short list of HCs for the study's monitoring program.
3. To optimize site sampling techniques and analytical methods.

The monitoring program was conducted in Phase 2 and the objectives were:

1. To estimate the present sewage treatment process efficiency.
2. To determine the factors influencing HCs removal:

e.g. i) treatment process operating variables such as solids retention time (SRT),
ii) the levels of recycled HCs in the plant, and
iii) seasonal effects such as Spring run-off and dry weather periods.

Phase 1 - Contaminant Selection

The parameters selected for monitoring included PAHs, total PCBs, pesticides, heavy metals and conventional pollution parameters.

This study is still in progress, and therefore, the study procedures and preliminary results for the eight hazardous organic compounds shown in Table 2 will be presented.

TABLE 2. EIGHT HAZARDOUS CONTAMINANTS MONITORED AT THE WPCP

COMPOUND	CHEMICAL CLASSIFICATION
Acenaphthylene Benzo(a)pyrene Fluorene Fluoranthene Naphthalene Pyrene	Polynuclear Aromatic Hydrocarbons (PAHs)
Carbazole Dibenzofuran	Polynuclear Heterocyclic Compounds

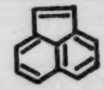
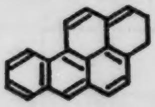
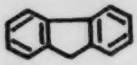
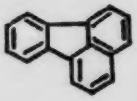
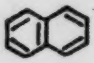
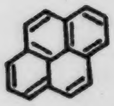
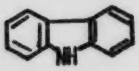
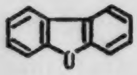
The chemical structures together with some physical data are provided in Table 3.

Environmental Significance

The environmental significance of these compounds can be summarized briefly as follows:

Although carbazole and dibenzofuran are not generally considered to be PAHs, they are structurally and chemically related to this group, as shown in Table 3. PAHs have been designated as priority pollutants by the U.S. EPA and in Canada they are included in the Water Pollution Control Directorate (WPCD) list of suspect/priority toxic chemicals. PAHs are probably the most widespread of all environmental contaminants (Jones and Leber 1979) and they have been identified in both Canadian and U.S. municipal treatment plants (Bridle 1982 and EPA 1982a). This group of compounds are non-polar and bioresistant. They have an affinity for lipids, and therefore, they tend to bioaccumulate (Zedeck 1980). Some PAHs have been shown to be carcinogenic in laboratory studies (Thakker et al 1979), and yet although structurally similar, they have widely varying degrees of carcinogenicity (Daniel et al 1979). Benzo(a)pyrene is an important member of this class of compounds which has produced cytotoxic, mutagenic and carcinogenic effects (Jones et al 1979).

TABLE 3. STRUCTURAL AND PHYSICAL DATA FOR EIGHT HAZARDOUS CONTAMINANTS (EPA 1979)

COMPOUND NAME	COMPOUND STRUCTURE	MELTING POINT °C	BOILING POINT °C	VAPOUR PRESSURE AT 20°C torr	SOLUBILITY IN WATER AT 25°C mg/L	LOG OCTANOL/ WATER PARTITION COEFFICIENT
Acenaphthylene		92.3	265.8	10^{-3} - 10^{-2}	3.93	4.33
Benzo(a)pyrene		177	495	5 x 10^{-9}	0.0038	6.04
Fluorene		116-7	293-5	10^{-3} - 10^{-2}	1.98 1.69	4.18
Fluoranthene		111	375	10^{-6} - 10^{-4}	0.26	5.33
Naphthalene		80.5	218	0.0492	34.4 31.7	3.37
Pyrene		156	393	6.85 x 10^{-7}	0.14 0.132	5.32
Carbazole		247-8	355	N.A.	N.A.	N.A.
Dibenzofuran		86-7	287	N.A.	N.A.	N.A.

Notes: NA = Data Unavailable

STUDY PROCEDURES - Phase 1 and Phase 2

Sampling Methods

Twenty-four hour composite samples were collected on two occasions in Phase 1 and during three separate periods in Phase 2, as shown in Table 4.

TABLE 4. PHASE 1 AND PHASE 2 SAMPLING PROGRAM

PHASE	SAMPLING PERIOD	NUMBER OF SAMPLING DAYS
1	August - September 1982	2
2		
Winter	December 1982 - January 1983	6
Spring	April and May 1983	4
Summer	June - August 1983	4

The sampling locations in the WPCP are shown in Figure 1, and the following samples were collected:

1. Combined influent; i.e. raw sewage combined with the in-plant recycle stream.
2. Effluent.
3. Waste activated sludge (WAS).

The in-plant return stream was also sampled on an intermittent basis. The WPCP operation was observed closely during the sampling periods and the process data together with observations of sustained WPCP operational upsets were recorded for correlation with the analytical results for the parameters being measured.

This study was unique, in that the solid and liquid fractions of the samples were examined separately. Therefore, the samples were centrifuged to separate the two fractions ready for analysis. However, the suspended solids concentration of the effluent was extremely low, and in order to avoid centrifuging very large volumes of effluent, WAS solids were used instead.

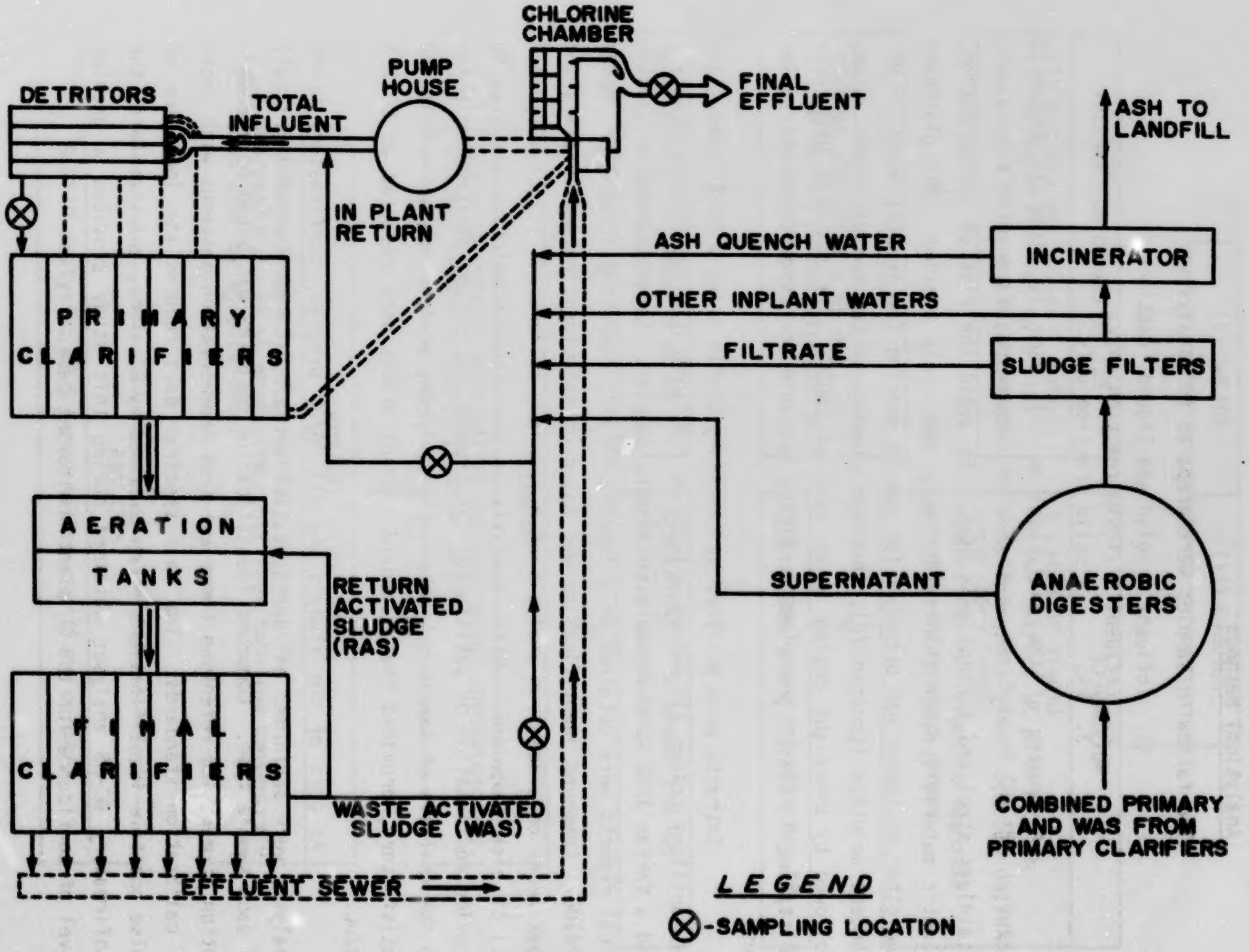

DETRITORS
TOTAL INFLUENT
PUMP HOUSE
CHLORINE CHAMBER
FINAL EFFLUENT
ASH TO LANDFILL
IN PLANT RETURN
ASH QUENCH WATER
INCINERATOR
PRIMARY CLARIFIERS
OTHER INPLANT WATERS
FILTRATE
SLUDGE FILTERS
AERATION TANKS
RETURN ACTIVATED SLUDGE (RAS)
SUPERNATANT
ANAEROBIC DIGESTERS
FINAL CLARIFIERS
WASTE ACTIVATED SLUDGE (WAS)
COMBINED PRIMARY AND WAS FROM PRIMARY CLARIFIERS
EFFLUENT SEWER
LEGEND
⊗-SAMPLING LOCATION

FIGURE 1 — WPCP PROCESS FLOW SCHEMATIC AND SAMPLING LOCATIONS

Analytical Methods

The following samples were prepared for analysis:

1. Influent - solid and liquid fractions.
2. Effluent - liquid fraction only.
3. WAS - solid fraction only.

A summary of the analytical methods used is given in Figure 2 (CANVIRO 1983b). Only equipment made of recommended materials (e.g. glass, stainless steel and teflon) were used. To avoid the risk of contamination, plastic materials other than teflon were completely avoided. All glassware and other equipment was prepared for use by soaking for several hours in detergent solution (Contrad 70), thorough rinsing in tap and distilled water, followed by overnight drying in an oven at 150°C. The solvents used were "Distilled in Glass" grade and certified suitable for chromatographic analyses.

Extracts were analyzed by capillary GC-FID using a DB-5 fused silica capillary column (15 meters x 0.25 mm I.D. with 0.25 um film thickness), and a Varian 3700 GC equipped with an auto sampler. Chromatograms and analytical reports were obtained on a Spectra-Physics computing integrator, Model SP4100. Compound identification was based on coincident retention times and peak areas obtained from analysis of a standard calibration solution (50 ng/uL) of each component. Daily analysis of the standard solution was used to provide confirmation of satisfactory instrument and column performance prior to the analysis of sample extracts. The equipment performance was considered satisfactory, provided that standard concentrations were reported as 50 ± 5 ng/uL.

As part of the study's overall QA/QC program, confirmatory GC-MS analyses were performed on representative extracts (18 percent of the total) of each sample type. Compound identification by GC-MS was based on a combination of matching retention times and mass fragmentation patterns with those of calibration standards. The mass spectral data reduces the incidence of false positive identifications often obtained by GC alone, and therefore, the confirmatory GC-MS analyses obtained during this study provided a greater level of confidence for the GC plant monitoring data.

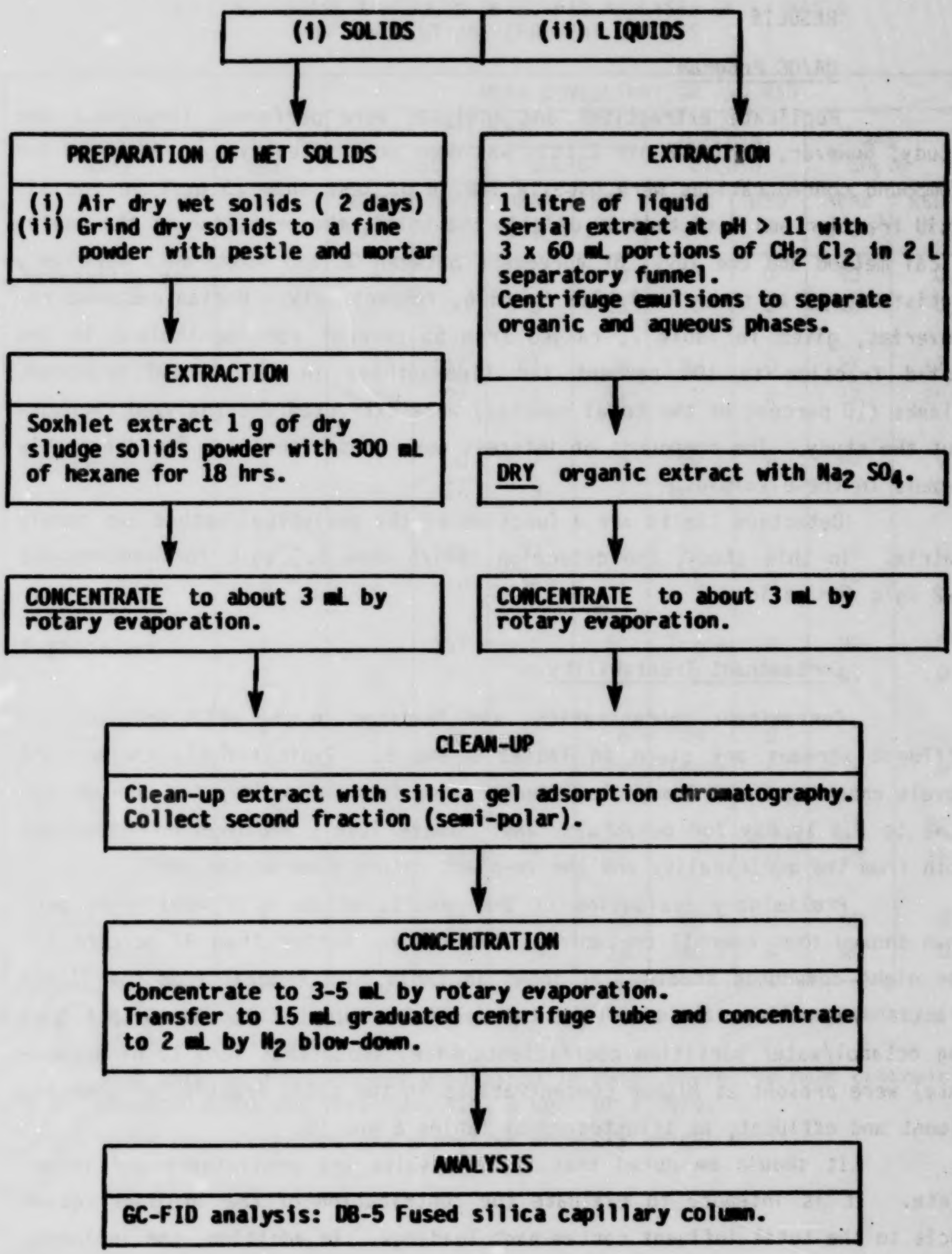

(i) SOLIDS
(ii) LIQUIDS
PREPARATION OF WET SOLIDS
(i) Air dry wet solids (2 days)
(ii) Grind dry solids to a fine powder with pestle and mortar
EXTRACTION
1 Litre of liquid
Serial extract at pH > 11 with 3 x 60 mL portions of $CH_2 Cl_2$ in 2 L separatory funnel.
Centrifuge emulsions to separate organic and aqueous phases.
EXTRACTION
Soxhlet extract 1 g of dry sludge solids powder with 300 mL of hexane for 18 hrs.
DRY organic extract with $Na_2 SO_4$.
CONCENTRATE to about 3 mL by rotary evaporation.
CONCENTRATE to about 3 mL by rotary evaporation.
CLEAN-UP
Clean-up extract with silica gel adsorption chromatography.
Collect second fraction (semi-polar).
CONCENTRATION
Concentrate to 3-5 mL by rotary evaporation.
Transfer to 15 mL graduated centrifuge tube and concentrate to 2 mL by N_2 blow-down.
ANALYSIS
GC-FID analysis: DB-5 Fused silica capillary column.

FIGURE 2: SUMMARY OF THE ANALYTICAL METHODS

RESULTS

QA/QC Program

Replicate extractions and analyses were performed throughout the study; however, during Phase 1 this was done more intensively. Although the compound concentrations were usually low; e.g. less than 15 ug/L in the liquid fraction and less than 50 ug/g in the solid, the precision of the analytical method and the level of agreement between GC and GC-MS data were very satisfactory, as shown in Tables 5 and 6, respectively. Median compound recoveries, given in Table 7, ranged from 65 percent for naphthalene in the solid fraction to 106 percent for fluoranthene in the liquid fraction. Blanks (10 percent of the total samples) were extracted and analyzed throughout the study. The compounds of interest were consistently at non-detectable levels in these samples.

Detection limits are a function of the analytical method and sample matrix. In this study, the detection limits were 0.5 ug/L for liquids and 1-2 ug/g for solids.

Contaminant Treatability

Contaminant concentrations and loadings in the WPCP influent and effluent streams are given in Tables 8 and 9. Typical daily contaminant levels entering the primary clarifiers ranged from 0.7 kg/day for acenaphthylene to 7.9 kg/day for benzo(a)pyrene. These levels included contributions both from the municipality and the in-plant return flow at the WPCP.

Preliminary evaluation of the results of the WPCP monitoring program showed that overall contaminant removal was better than 97 percent for the eight compounds studied, as shown in Table 10. Removal from the liquid fraction was much better than from the solid fraction. Compounds with higher log octanol/water partition coefficients (i.e. those that tend to bioaccumulate) were present at higher concentrations in the solid fraction of both influent and effluent, as illustrated by Tables 8 and 10.

It should be noted that these results are preliminary and incomplete. It is intended to evaluate the contribution of the in-plant return cycle to the total influent contaminant loadings. In addition, the influence of plant operating variables and seasonal conditions on treatment performance will also be determined.

TABLE 5. PHASE 1 RESULTS OF GC-FID ANALYSES OF EXTRACTS OF INFLUENT AND EFFLUENT SAMPLES

COMPOUND NAME	SAMPLING DAY	MEAN CONCENTRATION AND RSD							
		INFLUENT				EFFLUENT LIQUID		WAS SOLID	
		LIQUID		SOLID					
		MEAN ug/L	RSD %	MEAN ug/g [a]	RSD %	MEAN ug/L	RSD %	MEAN ug/g [a]	RSD %
Acenaphthylene	1	4	29	5	0	ND	0	ND	0
	2	5	20	5	25	1	0	ND	0
Benzo(a)pyrene	1	1	141	41	8	ND	0	27	17
	2	3	22	34	31	1	0	17	26
Fluorene	1	3	0	7	8	ND	0	ND	0
	2	4	16	9	7	Trace	0	1	172
Fluoranthene	1	3	35	37	2	ND	0	8	0
	2	3	0	44	7	1	0	14	8
Naphthalene	1	1	151	2	50	Trace	0	ND	0
	2	2	89	ND	0	1	0	ND	0
Pyrene	1	2	55	25	6	ND	0	7	8
	2	2	0	27	10	Trace	0	9	6
Carbazole	1	11	7	21	14	ND	0	11	22
	2	10	40	26	16	1	0	13	16
Dibenzofuran	1	2	29	4	16	ND	0	ND	0
	2	3	0	5	25	ND	0	ND	0

Notes: Each mean and relative standard deviation (RSD) is based on the results of three replicates.

High RSDs were occasionally obtained in cases where the mean contaminant concentration was very low; e.g. 1 ug/L or 1 ug/g.

a = Solid concentrations are expressed on a dry weight basis.

TABLE 6. COMPARISON OF RESULTS OBTAINED BY GC-FID AND GC-MS DURING PHASE 1 OF THE STUDY

COMPOUND NAME	SAMPLING DAY	CONCENTRATION							
		INFLUENT				EFFLUENT LIQUID ug/L		WAS SOLID ug/g	
		LIQUID ug/L		SOLID ug/g					
		GC	GC-MS	GC	GC-MS	GC	GC-MS	GC	GC-MS
Acenaphthylene	1	3	2	5	7	ND	ND	ND	Trace
	2	4	<10	4	4	1	ND	ND	ND
Benzo(a)pyrene	1	1	ND	38	23	ND	ND	26	16
	2	2	<10	36	5	1	ND	17	4
Fluorene	1	3	1	7	5	ND	Trace	ND	Trace
	2	4	<10	8	7	Trace	ND	2	ND
Fluoranthene	1	3	1	37	35	ND	Trace	8	7
	2	3	<10	43	47	1	ND	13	11
Naphthalene	1	ND	Trace	3	3	ND	Trace	ND	ND
	2	ND	ND	ND	1	1	<10	ND	1
Pyrene	1	2	Trace	24	19	ND	Trace	8	5
	2	2	<10	26	35	1	ND	9	13
Carbazole	1	12	NR	19	NR	ND	NR	12	NR
	2	14	NR	28	NR	1	NR	14	NR
Dibenzofuran	1	1	NR	4	NR	ND	NR	ND	NR
	2	3	NR	4	NR	ND	NR	ND	NR

Notes: GC analyses were performed by CANVIRO CONSULTANTS LTD.

GC analyses were performed on a Finnigan 1020 GC-MS at the Wastewater Technology Centre in Burlington, Ontario.

Solid concentrations are expressed on a dry weight basis.

NR = No result; compound not measured.

ND = Not detected.

Trace = Compound was detectable, but the concentration was too low to measure.

TABLE 7. SUMMARY OF RECOVERIES OF COMPOUNDS FOR SAMPLE SOLID AND LIQUID FRACTIONS

COMPOUND	SAMPLE FRACTION	PERCENT RECOVERIES		
		MEDIAN	20% [a]	80% [a]
Acenaphthylene	solid	82	75	88
	liquid	87	81	98
Benzo(a)pyrene	solid	78	58	83
	liquid	102	96	120
Fluorene	solid	89	78	92
	liquid	95	90	102
Fluoranthene	solid	88	80	96
	liquid	106	97	112
Naphthalene	solid	65	57	75
	liquid	80	68	86
Pyrene	solid	67	59	71
	liquid	102	96	105
Carbazole	solid	82	68	98
	liquid	102	86	107
Dibenzofuran	solid	82	78	90
	liquid	97	94	111

Notes:

Recoveries were determined at two levels:

i) 50 ug/g and 100 ug/g for solid samples.
ii) 50 ug/L and 100 ug/L for liquid samples.

Recovery data is based on 14 replicates for each sample fraction.

a = Recoveries were calculated at the 20 and 80 percentiles.

TABLE 8. MEDIAN CONCENTRATIONS OF COMPOUNDS IN INFLUENT AND EFFLUENT SAMPLES

COMPOUND	MEDIAN CONCENTRATION					
	INFLUENT			EFFLUENT		
	SOLID FRACTION ug/g	LIQUID FRACTION ug/L	% OF TOTAL DUE TO SOLIDS CONTRIBUTION	SOLID FRACTION ug/g	LIQUID FRACTION ug/L	% OF TOTAL DUE TO SOLIDS CONTRIBUTION
Acenaphthylene	6	2	32	Trace	ND	100
Benzo(a)pyrene	99	5	80	22	ND	100
Fluorene	8	2	50	Trace	ND	100
Fluoranthene	28	1	85	16	ND	100
Naphthalene	5	1	28	1	Trace	NC
Pyrene	27	1	85	21	ND	100
Carbazole	38	10	43	22	ND	100
Dibenzofuran	6	2	47	Trace	ND	100

Notes:

ND = Not detected

NC = Not calculated. Naphthalene in the liquid fraction was detectable, but the concentration was too low to measure.

TABLE 9. CONCENTRATIONS AND LOADINGS OF COMPOUNDS IN TOTAL INFLUENT AND EFFLUENT

COMPOUND	PARAMETER	TOTAL INFLUENT		TOTAL EFFLUENT	
		CONCENTRATION ug/L	LOADING kg/day	CONCENTRATION ug/L	LOADING kg/day
Acenaphthylene	Median	3	0.7	Trace	Trace
	No. of Detections	16/16		11/16	
Benzo(a)pyrene	Median	27	7.9	Trace	Trace
	No. of Detections	16/16		15/16	
Fluorene	Median	4	1.0	Trace	Trace
	No. of Detections	16/16		10/16	
Fluoranthene	Median	8	2.4	Trace	Trace
	No. of Detections	16/16		16/16	
Naphthalene	Median	3	1.1	Trace	Trace
	No. of Detections	16/16		12/16	
Pyrene	Median	7	2.1	Trace	Trace
	No. of Detections	16/16		16/16	
Carbazole	Median	19	6.2	Trace	Trace
	No. of Detections	16/16		15/16	
Dibenzofuran	Median	3	0.8	Trace	Trace
	No. of Detections	16/16		8/16	

Notes:
Trace Loading = Less than 0.1 kg/day (average plant flow = 300.000 m^3/day)
No. of Detections = Number of days detected during 16 day monitoring program.

TABLE 10. PERCENT REMOVAL OF COMPOUNDS BY WPCP TREATMENT

COMPOUND	MEDIAN PERCENT REMOVAL		
	SOLID FRACTION	LIQUID FRACTION	TOTAL
Acenaphthylene	97.5	100	99.9
Benzo(a)pyrene	71.1	100	98.7
Fluorene	98.6	100	99.9
Fluoranthene	49.1	100	97.8
Naphthalene	100	93.3	99.7
Pyrene	39.2	100	97.5
Carbazole	43.5	100	98.4
Dibenzofuran	99.9	100	100

CONCLUSION

Treatment Plant Performance

Trends in the treatment plant performance shown by these preliminary results are in good agreement with those obtained in the recent EPA 40 POTW study (EPA 1982a). Whilst PAHs were adsorbed onto the solids fraction, and therefore were largely removed during primary treatment, low levels of these compounds were measured in the WAS solids. Thus, whilst overall removability of these compounds by the WPCP was very good (>97 percent), detectable traces were discharged in the effluent (<.04 ug/L by estimation). On one isolated occasion during the sixteen days of the monitoring program for reasons yet to be identified, much higher levels than normal of these compounds (e.g. 413 ug/L compared to <25 ug/L of fluoranthene) were measured in the influent. On this occasion, slightly increased levels were observed in the effluent (e.g. 3 ug/L compared <1 ug/L of fluoranthene). This is in keeping with the EPA results (EPA 1982a), which suggest that the removal rate for most contaminants appeared to be fairly constant, a fixed percentage of the influent loading being removed during the treatment process.

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ISBN - 0 -7743-8797-1

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PART 2

AIR POLLUTION RESEARCH

CONSTELLATION HOTEL , TORONTO

NOVEMBER 29&30 , 1983

SPONSORED BY

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Acknowledgements

The Ministry of the Environment's Research Advisory Committee would like to acknowledge the cooperation and efforts of the authors and Ministry staff who contributed to the organization of the Conference.

The financial support of the Provincial Lottery Trust Fund for environmental health-related research in Ontario has largely contributed to the success of this program including its technology transfer.

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PART 2

AIR POLLUTION RESEARCH

Towards an Electrochemically-Based Chemoreceptor For Trace Atmospheric Organics

by

M. Thompson, U.J. Krull and A. Arya

Department of Chemistry, University of Toronto,
80 St. George St., Toronto, ON, M5S 1A1.

Prepared for presentation to the Ontario Ministry of the Environment, Research Advisory Committee, Technology Transfer Conference No. 4, Toronto, November 30, 1983.

ABSTRACT

Natural chemoreception provides a model for development of selective organic sensing where the basic premise of operation implies that transduction of a selective binding event occurs via change of electrochemical conductance features across sensing membranes. This work describes the construction and theory of an ammonia gas sensor based on an artificial bilayer lipid membrane (BLM) which was modified for selectivity with the antibiotic nonactin. Detection limits are presently in the 10^{-6} M range and selectivity coefficients for ammonia over methylamine and other similar organic species are extremely high. Presently limitations of sensitivity and selectivity are determined by the chemistry of the BLM. This has been investigated with respect to transmembrane ion conduction energy barriers and the alteration of the inherent membrane dipolar potential for development of a more generalized mechanism for signal transduction through employment of selective receptors. Preliminary results of Langmuir-Blodgett thin-film deposition technology for the production of stable organized membranes suitable for routine laboratory sensing will also be introduced.

INTRODUCTION

A significant majority of analytical measurements made today are concerned with the qualitative and quantitative analyses of organic species in complex matrices. The most common route for such analyses involves complete separation of sample components followed by identification and quantitation. However this process becomes increasingly difficult for complex samples and is unjustifiably expensive on both a temporal and financial basis if information is required for only a few components in the sample. This work attempts to investigate and evaluate the potential of an electrochemically-based detection system simulating that used by natural living systems for chemoreception with respect to selective organic sensing.

Advances of selective sensors have evolved dramatically over the last 15 years in the field of inorganic analysis. These sensors are based on the ion-selective electrode, the best known being the glass electrode which was initially developed in the 1920's. Analogous development of selective electrodes for organic compounds has also occurred over the last 10 years with varying degrees of success. Generally these systems are best described as hybrid electrodes, incorporating an inorganic ion-selective electrode measurement system with an organic selective membrane capable of generating the appropriate inorganic ion. The ion-selective electrodes encounter interference problems from ions of similar character, solubility dissociation or dissolution of sensing membranes and general ionic strength-ion activity relationships. The resulting potential is of mixed origin and generally limits detection of inorganic species to concentration ranges greater than 10^{-6} M. Organic selective electrodes have comparable detection limits since final measurement is performed by the inorganic ion-selective device. The interesting feature of the organic selective electrodes stems from the employment of complementary reactive enzyme-substrate or antibody-antigen pairs which establish selectivity. This has also been used to great advantage in immunoassay technology which represents another technique where selectivity is determined on the basis of reaction chemistry. However this system again requires separation techniques and is unsuitable for direct concentration determination. Considering this disadvantage and the poor detection limits of potentiometric electrodes, a distinct lack of a selective and sensitive electrochemical method for trace organic analysis without involvement of separation becomes obvious. Such technology is apparently employed by the chemoreceptive sensory systems of living organisms.

Interest in basic biochemistry has stimulated investigations of the chemoreceptive funnctions of natural living organisms. An overview of the mechanisms proposed for processes such as olfaction indicate that the electrogenic activity implicated in chemoreception at certain cell membranes provides an interesting model for the development of electrochemically baseed sensors(1). Such a direction is offered by the highly sensitive and selective interactions that occur between particular biological macromolecules, receptors and their complement ligands resulting in electrochemical, physiological and biochemical action at cellular localities. In order to study interactions at or across cell membranes, biochemists required a well characterized, easily prepared synthetic model resembling the structure and functions of natural systems. Such a structure, consisting of a planar bilayer of lipids was reported by Mueller et al.(2) in 1962 and has been termed the bilayer lipid membrane (BLM). These membranes of 6-8 nm thickness consist of two adjacent lipid monolayers oriented in a symmetrical but opposing manner as illustrated in Fig. 1. Chemically this structure has a central non-polar hydrocarbon region bounded on both sides with polar sheets of lipid headgroups which are hydrated in supporting aqueous electrolyte. A direct electrical potential applied across this structure will result in the passage of a small but finite ion current through the membrane. A typical current-voltage curve for a stable BLM has ohmic response over a range of approximately -60 to +60 mV with the current usually being on the order of 10^{-9} A/cm^2. Incorporation and membrane interaction with molecules or complexes which alter the standing transmembrane ion flux results in the production of an analytical signal. We have suggested that a membrane-bound receptor/stimulant interaction which could translate the binding event into a transmembrane ionic flux is a suitable model for the development of a sensitive and selective electrochemical sensor(1,3). A substantial number of substances will induce conductance changes in BLM. These include non-selective interactions with antibiotics and simple organic molecules as well as selective interactions of enzyme-substrate, antibody-antigen, hormone-receptor and olfactory receptor systems.

Since the basic premise for analytical signal generation is that a membrane embedded molecular compound or complex can in some way alter lipid matrix properties to influence transmembrane ion flux, it becomes important to establish which physical features of the membrane are responsible for limiting the ion current. Ion flux is governed by features such as membrane-external ion concentration, applied potentials and membrane internal fluidity, thickness, dielectric constant, surface charge and surface dipolar potential. Since typical BLM forming lipids contain a polar region with elements of differing electronegativity, it is anticipated that a net molecular dipole moment may exist. When lipid molecules are organized and packed into the BLM structure, such net dipoles concurrently become ordered and combine to create a plane of ordered dipolar charge. By virtue of this salient feature of a BLM, a net positive potential is generated and superimposed across membranes formed from neutral lipids(4,5). This electrostatic field is directly responsible for control of transmembrane ion flux and represents a static situation which can be perturbed to induce analytical signal generation. Two general mechanisms involving selective interactions are suitable for BLM stimulation eliciting an analytical response. These rely on either the creation of an ion conductive pathway within the membrane or on surface dipole or charge alteration, reflecting changes in either the chemical or electrical potential energy barriers respectively.

Application to Gas Sensing

Ideally a BLM gas sensing device will operate on the basis of selective interaction of a membrane embedded receptor with the small volatile organic of interest, resulting in a concentration dependent increase in conductance via one of the aforementioned mechanisms. The lack of knowledge in the area of receptor implementation and the present lack of suitable receptors restricts the employment of such a general mechanism. However specialized systems which demonstrate the usefulness of the lipid membrane matrix as a basis for receptor design can still be established.

Numerous conventional selective gas sensing electrodes are available for small molecules such as carbon dioxide, sulphur dioxide, nitrogen oxides, hydrogen sulphide and ammonia. All of these sensors rely on the same mechanism for signal generation, employing an optional external selective polymer membrane to control gas permeation into a thin film aqueous phase where solution equilibria cause substantial pH alterations which are readily measured with a glass electrode. Limitations for these systems often originate with solvent or electrolyte contamination problems, or are controlled by other competitive features involving the ion-selective elctrode or semi-permeable membrane. The BLM sensor does not succumb to the competitive effects which limit the use of ion-selective electrodes since the development of an equilibrium interfacial potential is not the primary source of analytical signal. In order to demonstrate the feasibility of gas detection with a BLM, a working prototype designed to measure ammonia gas has been developed. The general electrochemical and membrane support design characteristics are similar to those employed for a conventional glass electrode based ammonia sensor, but the glass sensing membrane normally employed for interfacial potential development is replaced by an ammonium conductive BLM.

This report will summarize the operation of the prototype BLM gas sensor and will describe the chemistry of the BLM from the standpoint of ion conduction for future implementation of the general receptor mechanism for selective detection.

INSTRUMENTATION AND EQUIPMENT

Electrochemistry

The electrochemical cell consisted of two identical machined perspex blocks separated by a teflon sheet (0.1 mm thickness) which contained a circular aperture (1 mm diameter) used for BLM support as illustrated in Fig. 2. An external direct potential was applied across the membrane between two agar salt bridge extended Ag/AgCl single junction reference electrodes (Orion Research Inc., Cambridge, Mass). The external circuitry consisted of a DC power supply and a microprocessor controlled multichannel digital electrometer (Keithley System 1, Keithley Instruments, Cleveland, OH) for data acquisition. An optical system consisting of a cold light halogen-quartz fiber optics illuminator and a wide angle twenty power microscope were used to investigate and monitor BLM formation for electrochemical studies. The solution cell and sensitive electronic equipment were isolated in a well grounded Faraday cage.

Capillary Gas Chromatography

A conventional packed column Varian Aerograph 2740-10 Gas Chromatograph (Varian Associates, Walnut Creek, CA) was modified for capillary gas chromatographic investigation of lipid and cholesterol oxidation. A glass lined splitter assembly, Inlet Splitter System (Chromalytic Technology P/L) including a septum purge and buffer volume to reduce gas viscosity effects replaced the conventional inlet. Stainless steel glass lined inlet and nickel outlet tubing was of 0.25 mm internal diameter and included a make-up gas tee junction at the end of the column to reduce dead volume effects. The detector was based on a sensitive flame ionization electrometer assembly employing hydrogen/air fuel mixtures and helium carrier and make-up gas. A separate gas flow controller (Pressure Control System, Chromalytic Technology) was used due to the low volumes of gas required. The capillary column employed for this work was a 30 m x 0.25 mm ID, Durabond fused silica column with DB-1 (equivalent to SE-30) 0.25 μm coating and a practical column efficiency of $N_{eff} = 100,000$ (J + W Scientific, Inc., Rancho Cordova, CA).

Gas Sensor

The basic design of the gas sensor electrochemical cell retained the concept of two solution compartments which were separated by a BLM. A BLM was formed in a standard teflon sheet, and was supported on one face by a conventional perspex half cell. A porous teflon semi-permeable membrane designed for use with an ammonia electrode (Ammonia Porous Membranes, Orion

Research Inc.) was used to trap a thin layer of electrolyte at the other membrane face and allowed interfacing to the gas phase. The trapped aqueous layer was connected by means of a thin channel to a Ag/AgCl reference electrode as seen in Fig. 3. A specially constructed external clamp was used to sandwich the teflon sheet and semi-permeable membrane to a central perspex sheet, allowing for easy apparatus cleaning and teflon replacement. External electrochemical equipment was oraganized as previously described.

PROCEDURES

Formation of BLM

The BLM were formed from solutions containing n-decane (which had been purified over an alumina column and dried over molecular sieves) and lipid/cholesterol in a 2%/2% weight-to-volume ratio. The lipid containing solution was first placed in an ultrasound bath for 1 minute to insure homogeneity and was then introduced to the 1 mm diameter teflon aperture by means of a fine sable hair brush. The resulting lipid plug trapped in the aperture between the two 5 ml aqueous solution compartments containing aqueous electrolyte would spontaneously thin to form a region consisting of a BLM, surrounded by a torus of excess lipid and solvent. (All experiments in this work employed 0.1 M KCl electrolyte and an applied potential of +25 mV unless otherwise stated). Observation of BLM formation could be directly accomplished through use of electrical monitoring of transmembrane charge passage or by surface optical reflectivity properties (6). Experiments requiring elevated temperatures were initiated as those described at room temperature. Stable BLM were prepared at 21 ± 1°C and then an infrared heating lamp was employed to increase the temperature at a rate of approximately 1°C /min.

Sample Preparation for Chromatography

Phospholipid modification for TLC and gas chromatographic analysis consisted of transmethylation to produce methyl esters of the lipid acyl chains. Numerous methods to accomplish this conversion have been described in the literature, though the best method for complete concentration independent conversion of small samples employs a base catalyzed reaction (7). Lipid samples of mass 1-10 mg were reacted for 20 minutes at 22°C in a reagent mixture consisting of 1 ml of freshly prepared 0.5 M sodium methoxide and 1 ml of dry benzene. Samples were then extracted with 3 ml of diethyl ether and washed with 3 ml of 1 M aqueous sodium chloride twice. The remaining diethyl ether/benzene extract was then dried over anhydrous sodium sulphate, concentrated with a nitrogen gas stream and was submitted to the gas chromatograph. Lipid/sterol decane solutions (suitable for BLM preparation) of 50-100 µl volume were similarly prepared without component preseparation for chromatographic analysis. Optimum column operating conditions are provided with the chromatogram shown in Fig. 10.

Gas Sensing Device

The operation of the gas sensor was based on an ion-carrying selectivity for ammonium ion of the antibiotic nonactin, coupled with the action of an ammonia gas semi-permeable membrane. Various electrolyte solutions based on KCl, LiCL and $MgCl_2$ were investigated for effectiveness in enhancing the BLM transmembrane ion current in the presence of standard aqueous concentrations of ammonium chloride at pH ∿ 4.0 in the presence of nonactin. Nonactin was added to one solution compartment whille the ammonium solution was introduced in the alternate aqueous phase. After determining the conditions for maximizing sensitivity to ammonium ion detection, one solution compartment was replaced with the gas phase interface allowing ammonia gas sampling.

RESULTS AND DISCUSSION

A BLM-Based Ammonia Gas Selective Detector

In order to demonstrate the feasibility of gas detection with a BLM, a working prototype designed to measure ammonia gas has been developed. The general electrochemical cell and membrane support design characteristics are illustrated in Fig. 3 and are similar to those employed for a conventional glass electrode based gas sensor. The operation of the system depends on a ammonia gas semi-permeable teflon membrane for the purpose of interfacing the fragile free BLM to the gas sample. This membrane offers a pre-selection of the analyte gas, however BLM selectivity to ammonia is maintained by incorporating polypeptides within thhe BLM which preferentially complex and carry ammonium ions rather than electrolyte cations.

Polypeptides which are recognized or classified as antibiotics demonstrate a remarkable ability to distinguish between chemically similar ions and selectively complex such ions for transport across BLM. Of the common antibiotic groups, only the valinomycins and the polyethers are very selective to Group I cations and also ammonium ion. The selectivity is a function of permeability constants. A summary of such constants is presented in Table 1 and contrasts the extreme selectivity differences between gramicidin, valinomycin, nonactin and a polyether. To reduce the background current measured for the gas sensor and to maximize selectivity towards ammonium ion, a polypeptide from the macrotetralide group such as nonactin sould be given preference. It should be incorporated and operated in a BLM supported by low concentrations of an electrolyte such as LiCl which is associated with extremely low residual current to minimize background conductance and maximize the competitive advantage for antibiotic binding of ammonium cation. Note that the concentration of antibiotic also determines the overall analytical signal. Maximum effect is attained when the antibiotic has a great selectivity for NH_4^+ over supporting electrolyte cation, and when the latter is maintained at the minimum concentration required for stable BLM formation. An ammonium ion concentration-response curve for nonactin in BLM bathed with 10^{-3}M and 10^{-4} M LiCl is shown in Fig. 4. As expected from the difference in sellectivity of thesee cations, $K_{NH_4^+}/K_{Li^+} \sim 10^4$, the limit-of-detection for this system is 10^{-6}M and compares favourably to the commercial ammonia electrode. Any positive deviation from a linear response curve may be due to the change in ionic strength associated with high concentrations of NH_4Cl. Greater selectivity may be attainable for the purpose of creation of more sensitive ammonium BLM sensors by appropriate structural synthesis and employment of crown ethers.

The application of the nonactin/LiCl system in the configuration of Fig. 3 to detect ammonia gas in a bulk gas phase has produced the results shown in Fig. 5. The results are time dependent as expected for a diffusion controlled gas permeation which results in the production of an ammonium ion population.

Model of BLM Gas Sensor Operation

In order to evaluate the influence of various design parameters on the described BLM gas sensor, a mathematical model considering ammonia gas and ammonium ion diffusion through the experimental system has been developed. The model which was used for these calculations is summarized in Fig. 6 and illustrates the various diffusion zones. For simplicity, the area chosen for the calculations was determined by the area of the BLM used in this work. This implies that the calculated current data represents a minimum analytical signal since lateral diffusion characteristics are not considered. Experimental data is developed in Fig. 7 to illustrate the trend of observed ion current as a function of the interfacial concentration of ammonium ion. Note that the efficiency of the ion transport process apparently increases as a function of the available interfacial ammonium ion concentration. This may be due to a concerted effect caused by reduction of membrane fluidity due to the order perturbing influence of nonactin, which is concurrently associated with an ion transport kinetic rate increase. An analysis of the influence of the thickness of the teflon membrane and underlying aqueous thin film indicates that the aqueous phase is the diffusion rate-determining zone and controls the final BLM ion current.

Analytical Potential

The sensitivity of the modified BLM towards ammonium ion compares favourably with the commercial form of the ammonia gas sensor. Treatment of nonactin loaded membranes with up to 0.005M methylamine hydrochloride did not produce any significant analytical signal, nor did it affect subsequent analysis of ammonium ion concentration, indicating a substantial selectivity advantage for the BLM based sensor. This is necessarily the case due to the origin of selectivity in these membranes. Since nonactin complexes the cation in the center of a large pseudospherical structure, and the cavity at the interior of this structure determines binding capability (6), it would be physically unfavourable to complex methylammonium ion compared to the smaller Group I and ammonium ions. Since methylamine is the most potent common interferent of the conventional ammonia gas sensor, and also is physically the smallest interferent, it would seem that the BLM sensor is effectively immune to interference from volatile amines. The selectivity of the modified BLM ammonia gas sensor therefore indicates a substantial technological advantage over conventional sensors. The solution experiments indicate that 99% of the final signal can be obtained for decade changes in ammonium ion concentration over a period of 1 to 2 minutes, implying that these electrodes are adequate for routine laboratory use when considering time criteria (8). Calculations indicate that the same holds true for gas phase sensing with BLM.

This prototype BLM-based gas sensing system illustrates technical deficiencies with design and function:

- the large volume of trapped aqueous electrolyte must be reduced to minimize response time and maximize analyte ion concentration

- transmembrane pressure transients and osmotic pressure differences must be controlled or eliminated

- a more efficient method of coupling the external electrode must be devised

- the influence of ion complexing agent in the trapped solution compartment must be evaluated

- the electrochemical response should be measured on the basis of integration of transmembrane charge passage since an equilibrium of partial gas pressure with bulk sample solution cannot be attained due to the dynamic ion transport system invoked for this work

- the semi-permeable teflon membrane used for BLM protection and work in aqueous solution should be removed when analyzing in the gas phase

- the BLM must be supported or altered in some manner so that it can achieve greater mechanical stability and still retain its electrochemical sensitivity.

However, these deficiencies do not preclude the further development of useful BLM-based selective gas sensors. These systems will eventually offer access to a previously unexplored realm of analysis when receptors suitable for binding of more complex gaseous organic species become available.

LIPID MEMBRANE DIPOLE PERTURBATION FOR SELECTIVE SENSING

Origins of Membrane Surface Dipole Potential

The origins of the dipolar potential can be traced directly to the intrinsic dipoles found in the lipid membrane polar regions. This work deals entirely with the lipid phosphatidyl choline (PC) and, therefore, with dipoles originating from carbonyl and Phosphorous-Nitrogen (P-N) species, though similar explanations can be applied for most lipids. A substantial body of information regarding the orientation of these dipoles in synthetic lipid membranes has been collected from nuclear magnetic resonance (9,10), X-ray and neutron diffraction (11,12), and infrared spectroscopic studies (13) indicating that the P-N vector lies approximately parallel to the membrane surface. Since the net dipole of interest is that quantity perpendicular to the membrane plane, these results imply that the majority of the dipolar charge is contributed by the two carbonyl moieties. Further contributions to the net dipole originate from water molecules which are present due to headgroup hydration (14). Direct measurements of lipid monolayer dipolar potentials at air/water (15) and hydrocarbon/water (16) interfaces using radioactive ionizing and vibrating plate electrodes have indicated that the dipolar contributions of the carbonyls, P-N and water vectors are approximately 68%, 16% and 16%, respectively. The actual net perpendicular dipole magnitude is much more difficult to obtain due to angular orientations, opposing dipoles and subsurface phenomena (17), but has been previously estimated to be of the order of 0.5 to 0.7 Debye per molecule of PC (15). The effect of aligned and packed lipid dipoles is to create a positive transmembrane potential which for a monolayer can be hundreds of millivolts in size (15,18) and is presumably of greater magnitude for a BLM. A schematic representation of an intrinsic BLM dipolar potential is shown in

Fig. 8. The application of an external potential determines the direction of ion movement. A comprehensive treatment of the effects of dipolar potential on membrane ion permeability has been produced by Szabo (19), from which the following pertinent relations are noted:

A generalized Nernst-Planck diffusion equation representing ion current density, J, through the membrane can be expressed as

$$-J = zFD(x)c(x)\frac{d}{dx}\left[\frac{\overset{\circ}{\mu m}(x)}{RT} + \frac{zF\Psi(x)}{RT} + \mathrm{Ln}c(x)\right] \qquad (1)$$

assuming a single ion is present in the membrane, where $c(x)$ is the permion concentration and

z = valence of permion

F = Faraday constant

x = position in membrane

$D(x)$ = diffusion coefficient

R = gas constant

T = absolute temperature

$\mu_m^\circ(x)$ = position dependent free energy

$\psi(x)$ = dipolar potential

This equation can be manipulated to lead to the important relation,

$$J \alpha \exp\left[\frac{-zF\Delta\psi_o}{RT}\right] \qquad (2)$$

where $\Delta\psi_o$ = change in potential between the membrane interior and the aqueous phase.

Similar expressions have been proposed and used by Haydon (18) and Lundstrom (20). It is assumed that considerable change in membrane current can be obtained by change of ψ_o through selective interactions at the membrane surface. The magnitude of the current change is determined by the initial value of ψ_o in addition to the total alteration of the potential.

We have successfully investigated electrochemically active receptor-like systems based on enzyme-substrate, antibody-antigen, hormone-receptor and polysaccharide-glycoreceptor interactions. Observations derived from non-selective membrane interactions with organic species known to alter dipolar potentials and from electrochemical dipolar potential probes, coupled with data from the latter receptor systems have established the existence of the dipolar potential alteration hypothesis as a plausible mechanism of receptor action. The fact that dipolar potential alteration is related to certain receptor activity indicates that this relatively simple yet highly selective mechanism should be pursued for practical receptor implementation.

Ion Conduction Through BLM

Few polarizable groups exist in the interior of a cholesterol/PC BLM, explaining the poor conduction properties which have been documented in the literature. The membrane forming solutions employed in this work were purposefully oxidized to create ion conducting membranes. This resulted in the introduction of polar species into the membrane interior, particularly through oxidation of the alkene residues found in the lipid acyl chains. The distribution of such double bonds in phosphatidyl choline can be combined with an estimate of polar group distribution of sterol to prepare an approximation of the probability density of polarizable groups distributed through the membrane. The probability that a molecule in a membrane will contain a functional group at a specified position has been calculated by assuming:

a) an estimate of lipid and sterol oxidation
b) a PC/C mole fraction ratio
c) a symmetric distribution of alkene groups about the center of the acyl chains in a typical divinyl methane structure
d) oxidation of the latter to form acyl chains with polar hydroperoxide substitutions

The simple model employed presents evidence for four localized high charge density areas suitable for ion binding or Born energy reduction through dipolar shielding effects. These areas are interspersed with a general "smeared out" increase of the internal dielectric constant due to the presence of polar groups.

Eyring has proposed an absolute rate theory of membrane ion permeation which treats the problem as a migration over a series of activation energy barriers (21). The size of the activation energy barrier to transmembrane ion permeation, the magnitude of reduction of this barrier by selective interactions and the location of the rate determining step to ion transport for the purpose of relocation and placement of a selective interaction must be determined to properly develop and maximize the analytical sensitivity of a BLM-based sensor. The relationship of unmodified BLM residual current to temperature provides thermal data suitable for analysis with the Arrhenius equation to determine the activation energy E_a :

$$\mathrm{Ln}(I_r) = \frac{-E_a}{RT} + \text{constant} \qquad (3)$$

where I_r = residual current, T = absolute temperature and R = appropriate gas constant. Figure 9 represents the data in standard graphical Arrhenius format, where the slopes of the resulting linear plots are proportional to the total activation energy barriers. The linearity of each plot is expected due to the small temperature range investigated. The barrier can be represented as an electrical potential by substituting the equation:

$$I_r = I_o \exp(-eQ/KT) \qquad (4)$$

where Q = potential energy barrier, e = standard charge and K = Boltzmann constant. This results in barriers of 1150 mV and 900 mV (error ± 5%) for mole fraction cholesterol contents of 0.66 and 0.80, respectively. The size of this barrier indicates that ions probably pass through the membrane in a partially hydrated form. The barrier is a function of both the electrostatic (estimated to be about 400-600 mV in magnitude by calculations and experiment) and the chemical potential energies, either of which may be reduced for signal generation. It is proposed that the oxidized sterol products will have the most influence on surface dipole potentials and lipid packing characteristics, while the oxidized alkene residues induce reduction of the hydrocarbon chemical potential energy barrier to ion flux.

Chromatographic and Thermal Investigation of Oxidation

The incorporation of cholesterol into BLM offers alteration of intra-membrane physical chemistry in addition to increased planar membrane strength and stability. The sterol can readily undergo an oxidative transformation to produce entities with increased polarity. At least 50 derivatives are involved in the autoxidation of cholesterol through processes involving light and heat (22), of which approximately 5-10 are produced in sufficient quantities to be deemed important. The complex mixture may contain substances of both membrane stabilizing and destabilizing influence though the former seems predominant. Analysis indicates that conversion of the cholesterol hydroxyl group and B-ring unsaturation to more polar moieties assists membrane stabilization. Formation and rearrangements of carbonyl, peroxide and hydroxyl residues at the A and B ring positions are common, and peroxides and other polar residues can form on the hydrocarbon chain (22). Generally oxidation initiation and propagation processes act on cholesterol to produce reactive intermediates such as hydroperoxides and cholest-5-ene-3-one, which eventually react to form the volatiles, alcohols, ketones, aldehydes, alkanes, olefins and acids commonly observed.

Thin layer chromatography is the most versatile simple analysis method suitable for general separation of the major oxidation products of both lipid and cholesterol. The results of the separation methodologies attempted indicate that under optimum conditions, up to three lipid and 15 cholesterol oxidation products can be qualitatively isolated and identified.

Quantification can be established on a relative basis of total sterol by techniques such as gas chromatography. The problems of separation encountered for mixtures of closely related sterol compounds are alleviated through implementation of high efficiency capillary columns. The inherent sensitivity of the flame ionization detection system is sufficient to measure nanogram quantities of the various sterols. For analysis, the sterol samples can be directly injected into the chromatograph in a solvent such as chloroform, though the thermal instability of the common cholesterol products at the temperatures required to vapourize the sample is such that often a thermal decomposition occurs (23).

A new method suitable for concurrent quantitative analysis of the extent of lipid and sterol oxidation has been developed during this work. The procedural details have been described in the experimental section, indicating that sample preparatiion does not include preseparation of lipid and sterol. The process provides volatile methyl esters of the lipid acyl chains which can be chromatographed with the oxidized sterols. At constant injection and column temperature, a reproducible chromatogram of lipid methylate, and native and pyrolyzed sterol products evolves as shown in Figure 10. An identification of the eluted species has been attempted through use of gas chromatographic analysis of TLC preseparated sterol products and the use of known standards. The results of this tentative identification are listed in Table 2. The development of a facile, rapid and quantitative technique for evaluation of sample oxidation provides the opportunity for correlation of electrochemistry with oxidation. This has been applied to test the hypothesis relating oxidation to electrostatic and chemical potential energy barrier alterations as determined by thermal electrochemical properties of BLM. The results of the initial studies are illustrated in Figure 11 and clearly indicate the validity of the hypothesis.

Note that oxidation of lipid/cholesterol predominantly occurs in the membrane forming solution rather than in the BLM. Investigation of the autoxidation of egg derived lecithin in the organic solvent chlorobenzene and as BLM vesicles in aqueous 0.1 M NaCl maintained at 30°C and one atmosphere pressure of oxygen confirmed that the reaction rate was greatly reduced in the aqueous phase (24). This was probably a consequence of the high viscosity of the BLM interior, resulting in a ratio of efficiency of chain initiation for oxidation of 0.66/0.043 for the organic/aqueous phases respectively (24). This indicates that the gas chromatographic method employed here is a suitable technique for quantitative evaluation of the oxidation products in experiments using BLM.

SUMMARY OF THE ANALYTICAL POTENTIAL OF BLM SELECTIVE SENSORS

Processes Controlling the Analytical Response

The previous section demonstrated the origins of ion conductive mechanisms which are required for signal generation in the absence of membrane intrinsic protein based pathways. The principle of selective sensing is most simply incorporated into the BLM by defining the receptor at the membrane surface. The receptor stimulant interaction should decrease an electrostatically controlled rate-determining ion transport barrier which limits transmembrane conduction by impeding ion access to some form of facilitated transport pathway. To further simplify the system, such a pathway should be based on intrinsic properties of the molecules which form the membrane (implying that control of chemistry is of paramount importance if reproducibility is to be attained). A more complicated electrostatically controlled process involving activation or regulation of protein based conduction systems is also feasible.

The BLM sensor can be considered a useful prototype of a selective trace organic sensor which has numerous advantages over conventional techniques such as biocompatibility, selectivity, low cost, ease of use and portability. The system can be sensitive and usually has a limit of detection of 10^{-6} M or better for simple non-selective membrane interactions. Receptor systems investigated in this work indicate detection limits of at least 10^{-8} M for the analyte of interest. Presently the lowest detection limit has been obtained for non-selective interaction with an ion transport complex known as valinomycin, and is in the range of 10^{-12} to 10^{-13} M. It is important to note that experimentally it is possible to integrate some agents such as valinomycin into BLM and thereby increase detection limits. Similarly, such a process makes it possible to measure the electrochemical response of one molecule if for example it has the capability to produce a single highly conductive transmembrane pore. The condition of integration places a new dimension on the concept of limit-of-detection since it becomes a time dependent factor, where previously it has only been concentration dependent. The minimum detectable signal for BLM sensors will always be determined by residual current magnitude controlled by intrinsic conductance properties and effects due to contamination. The techniques of polarography and Auger electron spectroscopy indicate that optimization of signals from BLM sensors may involve differentiation of the primary ion current response to eliminate background interference. Maximum detectable concentrations are determined by saturation of:

receptor sites, kinetic processes, membrane surface density or ion conductive pathways. Further control of sensitivity can be achieved through amplification processes coupling concerted alterations of chemical and electrical potential barrier decreases.

Sources of Selective Receptors

The design of receptors for BLM can be accomplished in numerous ways, the most useful of which involve:

a) extraction from natural sources as employed in this work
b) modification of existing enzyme-substrate or antibody-antigen systems
c) synthesis via biochemical and genetic engineering routes
d) synthesis from first principles.

Extraction of natural receptors can be a tedious time consuming process prone to failure. The receptor activity after chemical processing is usually decreased and can be easily destroyed. However this process has been the most successful route for obtaining active receptors in the laboratory. Many enzyme-substrate and antibody-antigen type systems exist but cannot be incorporated into BLM due to polarity, size or steric problems. Recent work has demonstrated that either protein component of such systems can be reacted with long chain hydrophobic molecules to form amide bonded acyl chains which assist membrane incorporation through hydrophobic interactions without substantially altering biochemical activity (25). The relatively new area of genetic engineering has made possible the development of a wide range of proteins on a reasonable production scale. Monoclonal antibodies may be the first of such manufactured proteins to be used in BLM.

One important aspect of this work is that some of the basic criteria of receptor structure, membrane position and mechanism of electrochemical action necessary for proper receptor function have been established. Presently more work is required in this area, but the feasibility of synthesizing receptors in the laboratory for specific targets has been demonstrated. This will become an area of substantial importance for gas sensing technology since the opportunity to finally obtain selective receptors for gases of interest has come of age.

Practical Implementation of Receptors

Since BLM are not practical matrices for routine laboratory use of selective sensors, the integration of receptor activity with stabilized ordered lipid films developed by preparative Langmuir-Blodgett deposition techniques is being investigated. It is anticipated that such deposition techniques for formation of appropriate stabilized films will become well developed over the next few years. The technique will require the use of a vibration free device for dip casting of lipid films onto a suitable substrate, followed by polymerization to stabilize the lipid structure. Knowledge concerning receptor positioning and function will be critical for designing a useful sensing membrane. Preliminary work has demonstrated that organized lipid films can be deposited on hydrogels, the latter being used to replace the present conventional solution compartments required for BLM support and the electrochemical cell. Microelectrodes implanted into the gel assist in device miniaturization and allow the sensing membrane to be employed in an identical operational configuration to that described for BLM in this work. Receptors embedded in stablized membranes which maintain the electrochemical advantages of the present system will allow testing and implementation of the first practical biosensors.

ACKNOWLEDGEMENTS

We are indebted to the Ontario Ministry of the Environment, Air Resources Branch for support of this work, and we would like to thank L.I. Bendell-Young and A. Azzopardi for their assistance with some of the experimental work.

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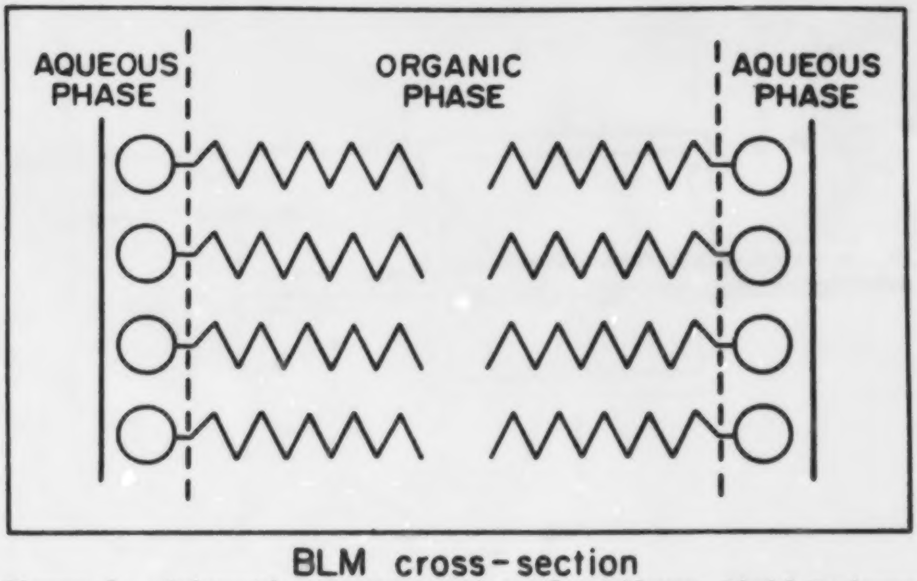
AQUEOUS PHASE
ORGANIC PHASE
AQUEOUS PHASE
BLM cross-section

Figure 1: Schematic representation of a Bilayer Lipid Membrane.

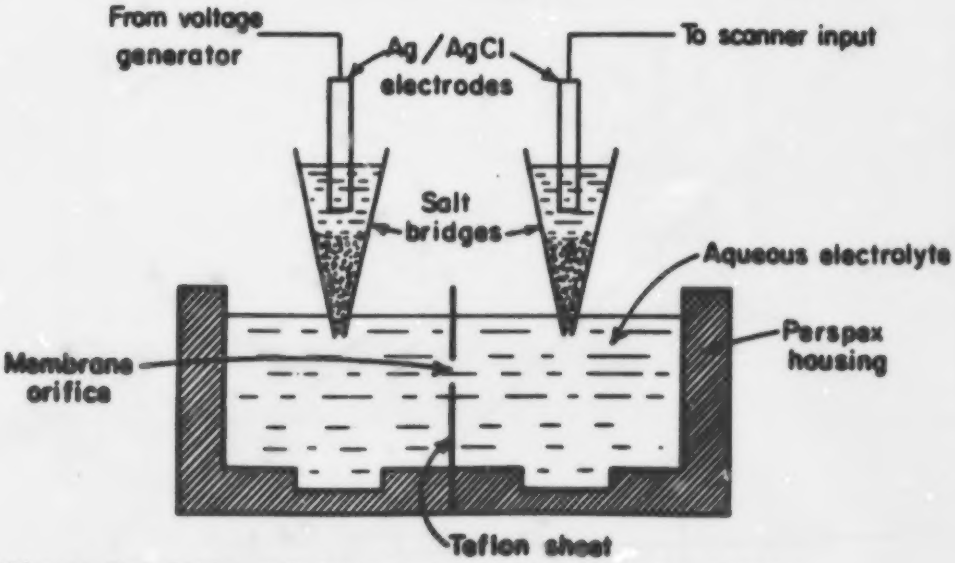
From voltage generator
Ag/AgCl electrodes
To scanner input
Salt bridges
Aqueous electrolyte
Perspex housing
Membrane orifice
Teflon sheet

Figure 2: Cell used for electrochemical study of BLM.

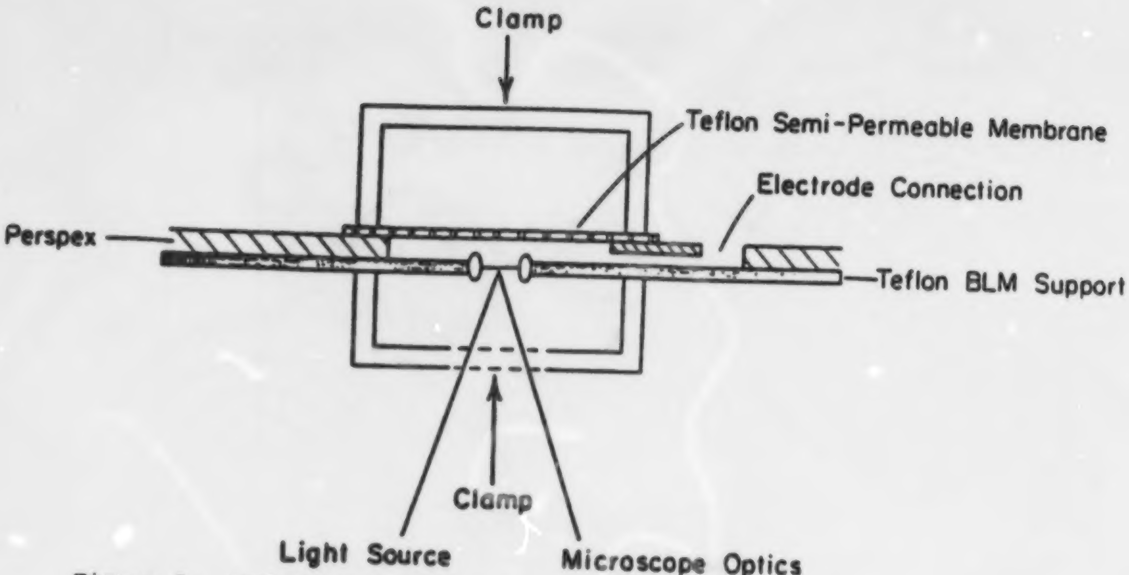
Clamp
Teflon Semi-Permeable Membrane
Electrode Connection
Perspex
Teflon BLM Support
Clamp
Light Source
Microscope Optics

Figure 3: BLM support system used in the ammonia gas detection cell.

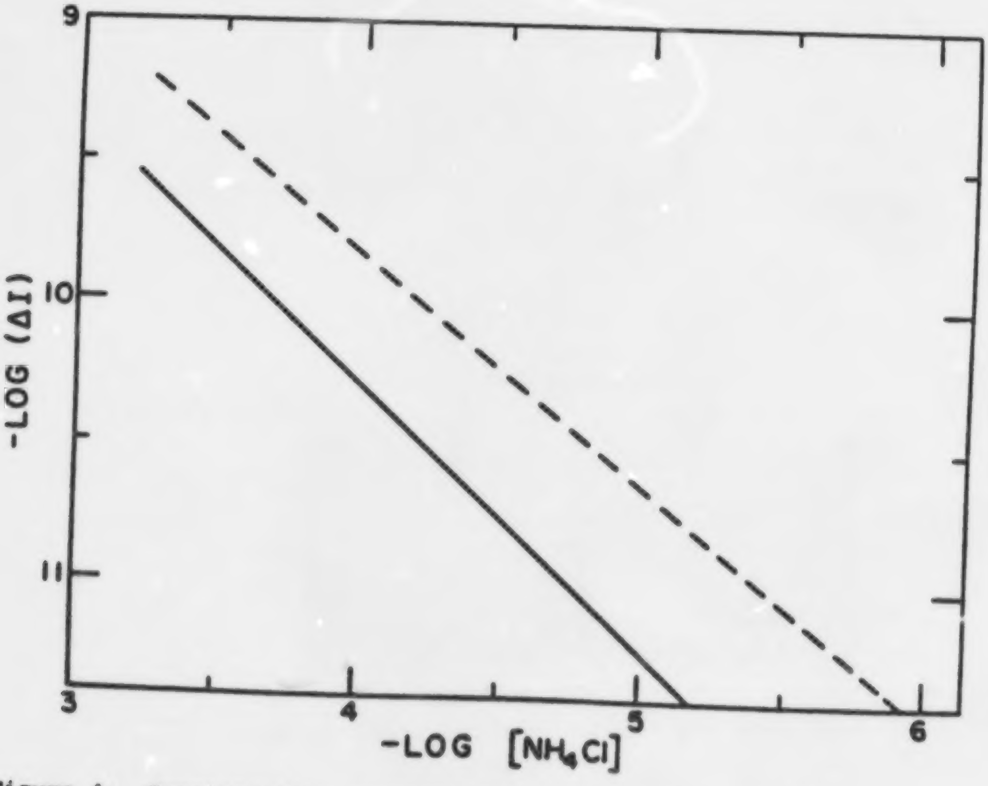

Figure 4: Concentration response curves for a nonactin/LiCl system to NH_4Cl. —— 10^{-3}MLiCl, ---- 10^{-4}MLiCl.

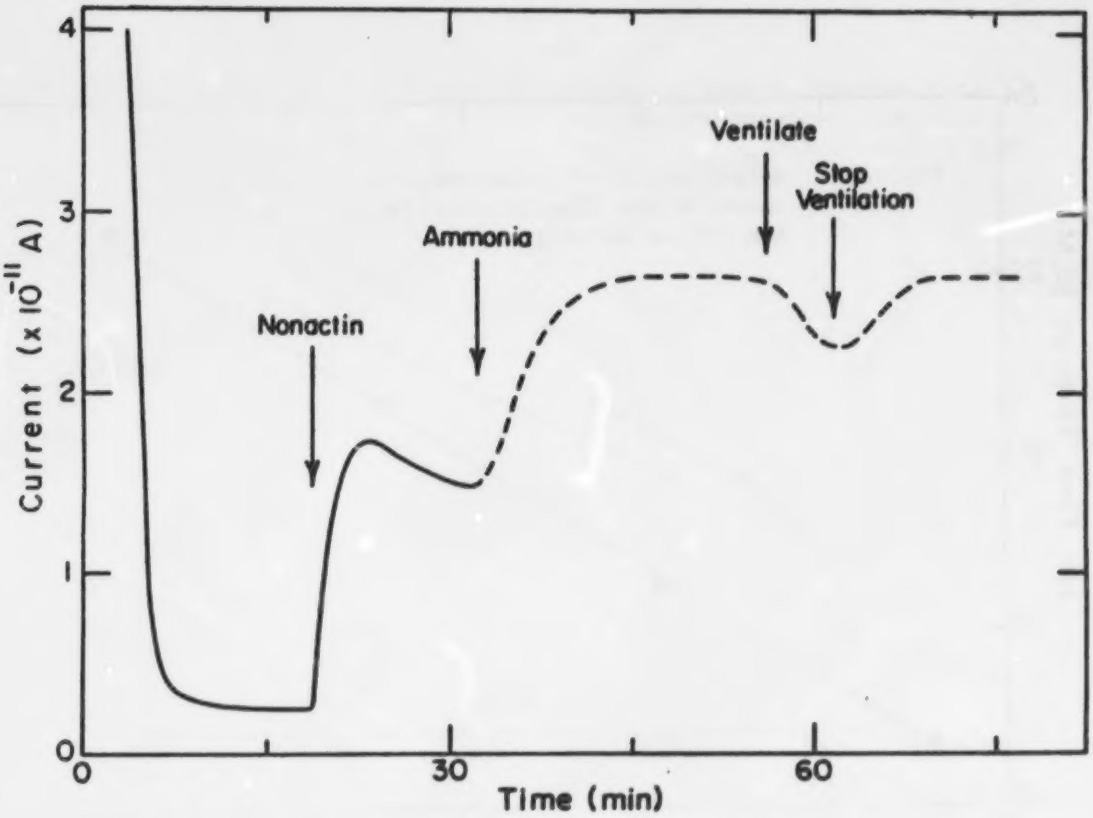

Figure 5: Sensing of concentrated ammonia gas with cell described in Fig. 3. (Nonactin = 10^{-3}M, LiCl = 10^{-4}M, +25 mV)

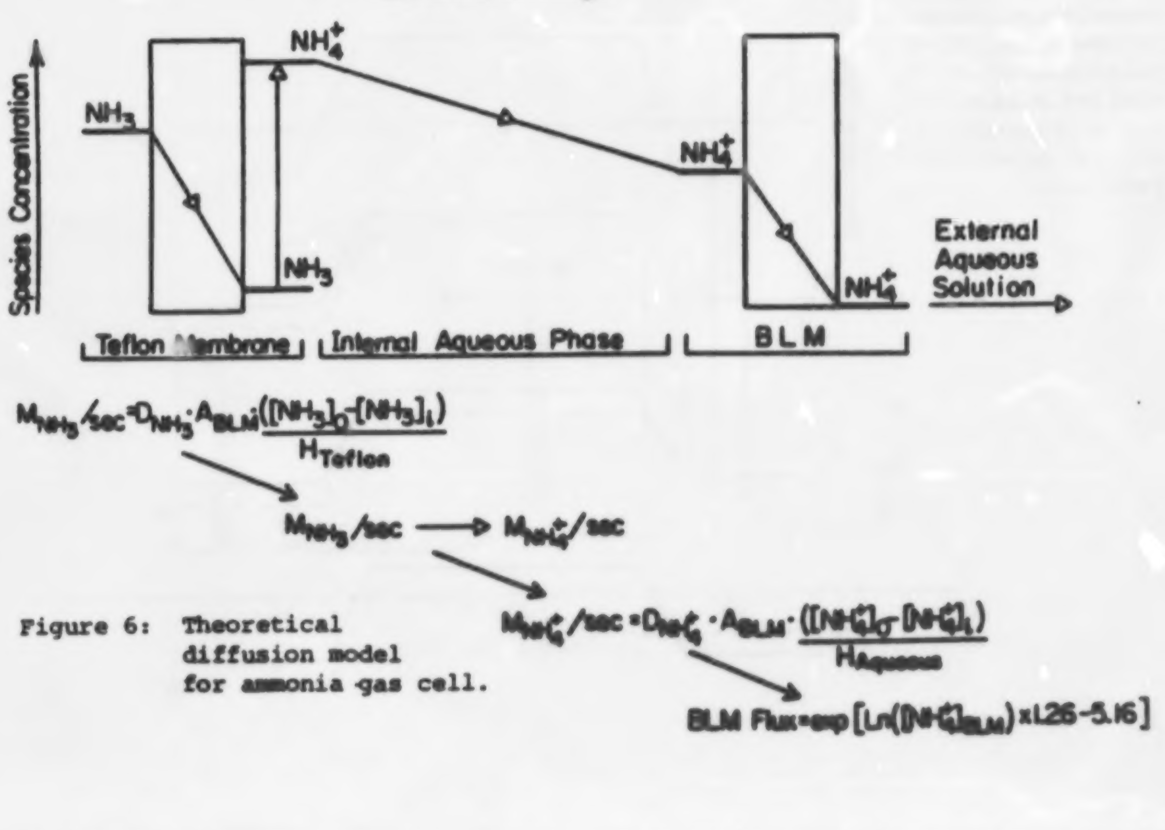
Distance
Species Concentration
NH$_3$
NH$_4^+$
NH$_3$
NH$_4^+$
NH$_4^+$
External Aqueous Solution
Teflon Membrane
Internal Aqueous Phase
BLM
$M_{NH_3}/sec = D_{NH_3} \cdot A_{BLM} \cdot \frac{([NH_3]_O - [NH_3]_i)}{H_{Teflon}}$
$M_{NH_3}/sec \longrightarrow M_{NH_4^+}/sec$
$M_{NH_4^+}/sec = D_{NH_4^+} \cdot A_{BLM} \cdot \frac{([NH_4^+]_O - [NH_4^+]_i)}{H_{Aqueous}}$
BLM Flux = exp [Ln([NH$_4^+$]$_{BLM}$) x 1.26 - 5.16]

Figure 6: Theoretical diffusion model for ammonia gas cell.

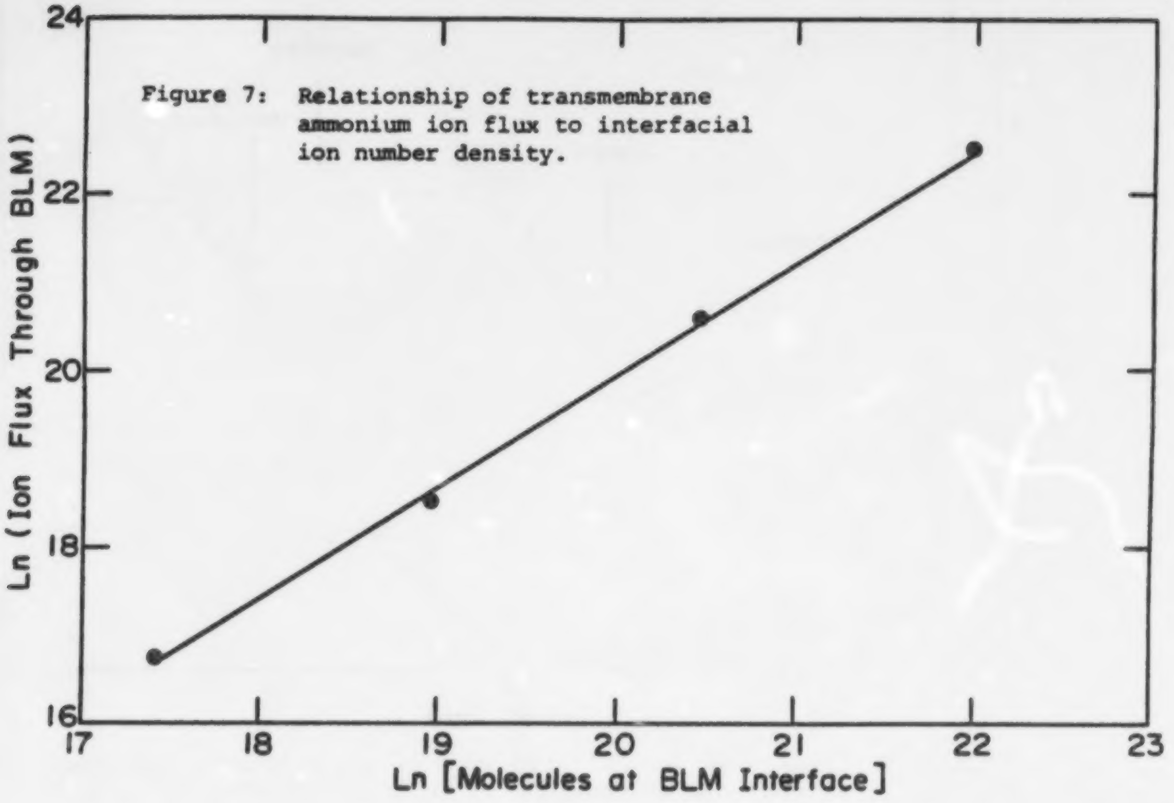

Figure 7: Relationship of transmembrane ammonium ion flux to interfacial ion number density.

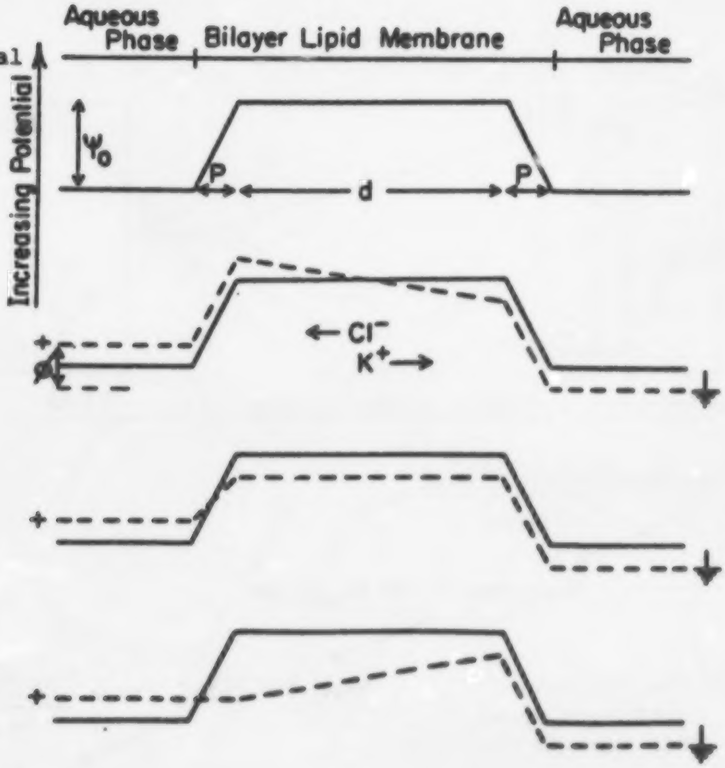
Aqueous Phase
Bilayer Lipid Membrane
Aqueous Phase
Increasing Potential
ψ_0
P
d
P
+
ϕ
← Cl⁻
K⁺→
+
+

Figure 8: Trapezoidal representation of transmembrane dipolar potential, applied potential and unilateral dipolar potential reduction.

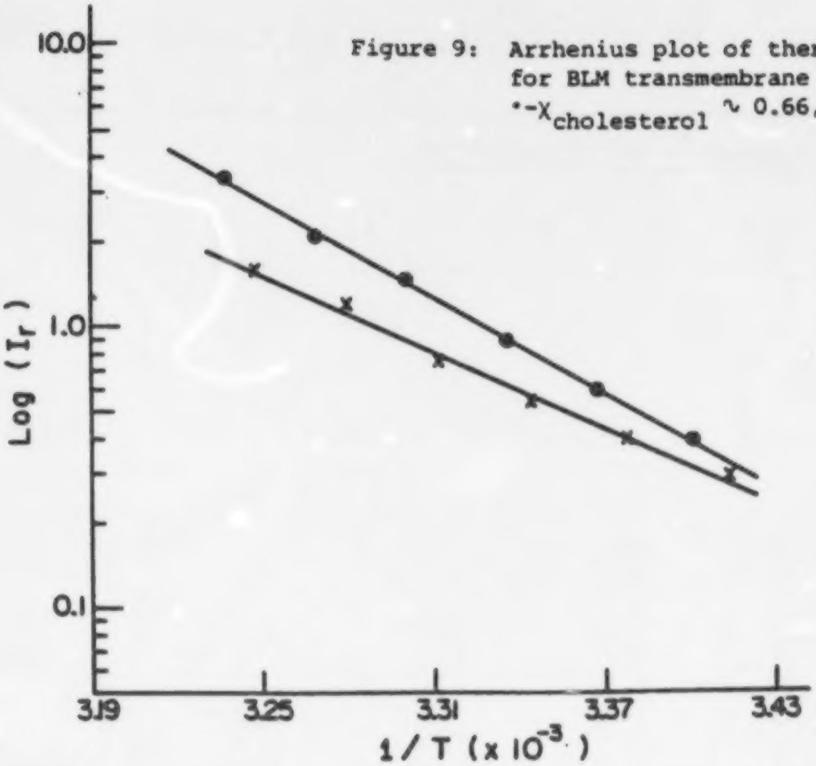

Figure 9: Arrhenius plot of thermal data for BLM transmembrane ion flux. •-$\chi_{cholesterol}$ ∿ 0.66, x-χ ∿ 0.80.

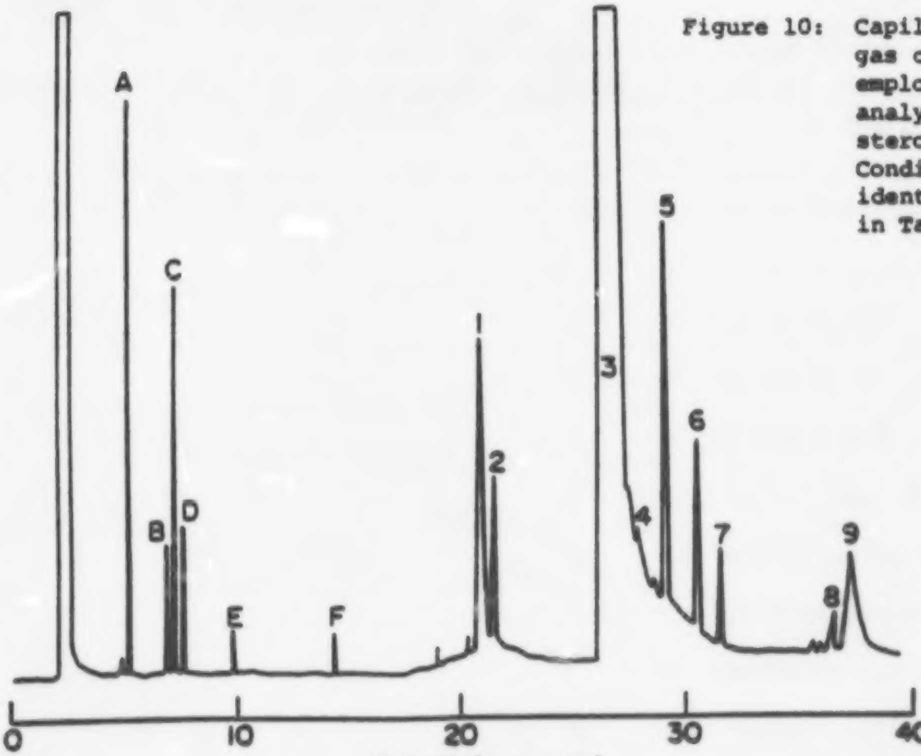

Figure 10: Capillary gas chromatogram employed for analysis of lipid/sterol oxidation. Conditions and identifications in Table 2.

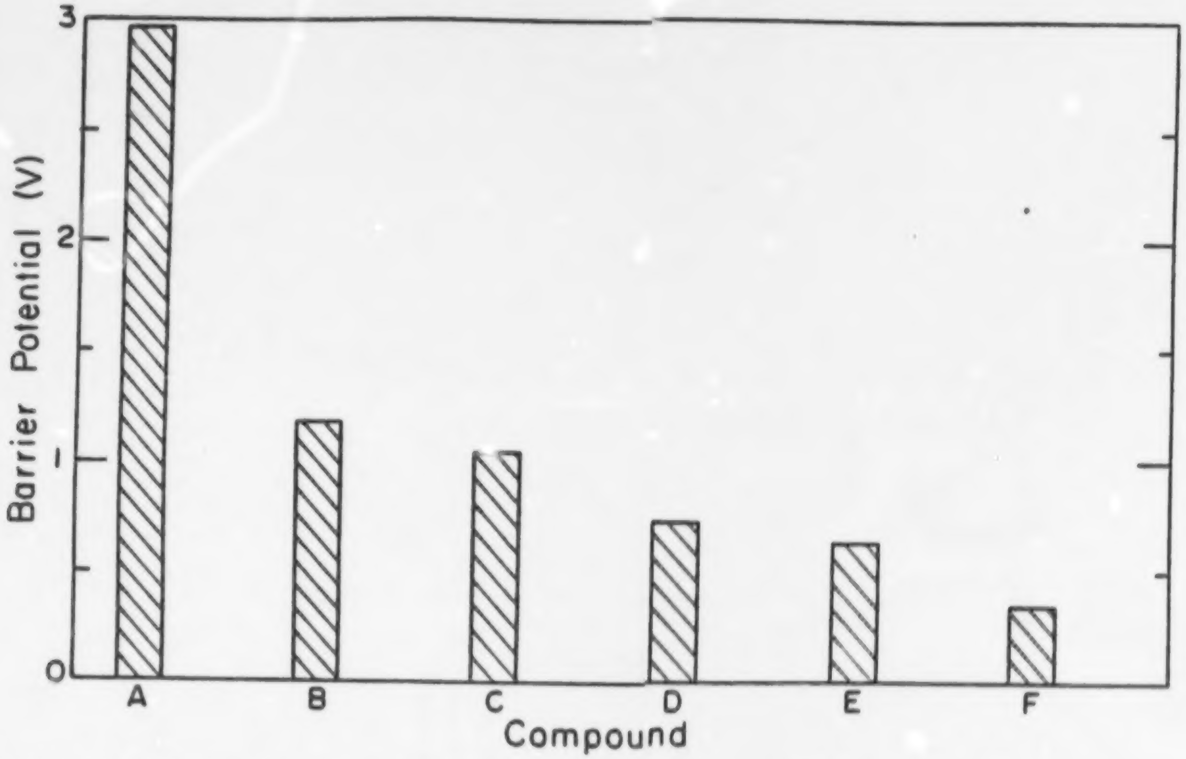

Figure 11: Arrhenius activation energy barrier to transmembrane ion flux as determined by cholesterol oxidation products ($\chi_{sterol} \sim 0.6$)

Compound	Identification
A	7α-hydroxycholesterol
B	cholesterol
C	5,7-cholestadien-3β-ol
D	5α-cholestan-3α,5,6β-triol
E	7-ketocholesterol
F	5,6-epoxycholestan-3β-ol

TABLE 1 : Relative Antibiotic Selectivities for Univalent Cations

Antibiotic	Log[Permeability X^+/Permeability K^+]			
	Li^+	Na^+	K^+	NH_4^+
Gramicidin	-0.9	-0.2	0	+0.1
Valinomycin	-6	-5	0	-2
Nonactin	-3	-2	0	+0.75
Crown Ether *	-2	-1.5	0	-0.5

---

*bis(t-butyl)dicyclehexyl-18-crown-6

Table 2: *Identification of products shown in capillary gas chromatogram of Fig. 10.

Lipid Acyl Methylate Product		Alkene Identification	Sterol Product		Tentative Identification
A	-	16:0	1	-	7α-hydroxycholesterol
B	-	18:2	2	-	5,6-epoxycholestan-3β-ol
C	-	18:1	3	-	cholesterol
D	-	18:0	4	-	cholesta-3,5-diene
E	-	20:4	5	-	cholesta-3,5-dien-7-one
F	-	22:6	6	-	5α-cholestane-3α,5,6β-triol
			7	-	5,6-epoxycholestan-3β-ol
			8	-	5α-cholestane-3α,5,6β-triol
			9	-	7-ketocholesterol

*Operating Conditions: Temperature programmed from 200 to 280°C @ 4°/min. from 0.5μℓ injection. Injector temperature ∿ 325°C. Helium carrier gas flow ∿ 0.5 ml/min.

METHODS FOR SAMPLING AND ANALYSIS OF ASBESTOS AIR POLLUTION IN ONTARIO

By

Dave K. Verma, Nancy E. Clark, and Jim A. Julian
Occupational Health Program
McMaster University
Hamilton, Ontario

Abstract

Asbestos, a known fibrogenic and carcinogenic material, has been identified in the past as the problem associated with occupational environments. It is estimated that almost half of all occupationally related cancers are caused by asbestos. Asbestos disease of clinical significance is probably undetectable at low levels of exposure. Some malignancies, particularly mesothelioma appear to be associated with surprisingly low levels of exposure. This accounts for the importance of asbestos pollution of outdoor air as well as of indoor air (eg. schools and offices) of Ontario.

This presentation will report on the ongoing research funded by the Ministry of the Environment's Air Resources Branch. The rationale for research issues will be discussed. Data on the examination of methods for sampling and analysis of low levels of asbestos including various electron microscopic techniques will be presented.

INTRODUCTION

Asbestos, a known fibrogen and carcinogen, has been identified in the past as a problem associated with occupational environments (factories, mines, construction, etc.) (1-7). It is estimated that almost half of all occupationally related cancers are caused by asbestos (8).

Diseases associated with asbestos are generally dose dependent. Some malignancies, particularly mesothelioma, appear to be related to surprisingly low levels of exposure accounting for the importance of asbestos pollution in outside air.

Because of its carcinogenic potential, asbestos contamination of outdoor air as well as indoor air (eg. schools and buildings) has now taken on a much greater importance. It is necessary that methods be developed and data collected to accurately detect and quantify asbestos in these situations so that the extent of the problem can be more precisely assessed. It is a well known fact that the present environmental guidelines for asbestos in Ontario and elsewhere (See Table 1) have been arrived at by extrapolation of occupational exposure data (9,10) and by comparison of outdoor background levels with those found in asbestos insulated buildings (11). The environmental guidelines are based on evaluations using electron microscopy.

In the occupational setting, health effects were related to the fibre levels obtained by traditional occupational exposure measurement techniques (the membrane filter method using optical microscopy, thermal precipitator counts, impinger counts, etc.).

The fibre counts obtained by phase contrast optical microscopy (PCOM) differ greatly from those obtained by electron microscopy. (EM). It has been suggested that counts obtained by electron microscopy could be 50 to 100 times higher than those counted using the optical method on the same air sample (12,13). In a recent study, a similarly high ratio of EM to optical fibre counts in air samples has again been demonstrated (14).

The issue of fibre dimension, that is, sizes visible by EM and those visible by PCOM, is shown graphically in Figure 1. This is extremely important if the carcinogencity of asbestos fibres is related to fibre morphology. The fibres that are thin (less than 1.5 µm) and longer than 8µm are suspected of being the most carcinogenic (15). A vast majority of such thin and long fibres could not be detected by optical microscopy but can only be seen by Electron Microscopy (See Figure 1). The proportion of fibres in the different size ranges depends on the type and operation involved. Furthermore, the PCOM method may include other non-asbestos fibrous material. Accurate identification and quantification of asbestos in outdoor air can only be reliably obtained using EM methods.

At present, data does not exist that relates low asbestos fibre levels obtained by EM to health effects. However, a grading of hazard potential can be developed if the following data is accumulated:

i) an inventory of fibre levels in outside air by EM,

ii) in selected occupational exposure situations, air samples analyzed by both EM and PCOM.

A correlation between EM and PCOM fibre counts could be established for specific occupational settings and fibre types. The health effects based primarily on optical counts could then be related to EM fibre counts. This is one feasible way of obtaining estimates of the risk associated with outside asbestos exposure (see Figure 2).

It is clear that in order to assess health risks associated with asbestos air pollution, the most important research tasks are:

i) establishment of relationships between fibre counts measured by the Phase Contrast Optical Microscope (traditional occupational method) to those assessed using the Electron Microscope for various operations and processes involving different types of asbestos.

ii) establishment of precise and accurate EM methods to evaluate asbestos air pollution. This should include a proper strategy for air sampling, sample preparation, counting, sizing and identification.

Sample Preparation

Initial research work was concentrated on setting up Electron Microscopic evaluation techniques. This involved looking at both direct and indirect sample preparation methods (Figure 3). Since we are interested in the size distribution of airborne asbestos fibres, we have concentrated on the direct transfer methods. In particular, we chose a modified Ortiz and Isom technique (29) as shown in Figure 3(B). We are using Millipore (mixed cellulose ester) rather than Nuclepore (polycarbonate) filters because of their ease of dissolution and better collection efficiency.

Fibre Counting Strategy

The next step was the establishment of our tentative strategy for counting fibres on the EM. Eight different EM counting strategies (16-23) from 3 countries (U.K., U.S.A., and Canada) were evaluated. All of the strategies were rejected on validity grounds. Most were based on non-random (ie. systematic) selection of counting fields. We have proposed an interim counting strategy that is based on truly random selection of grid openings on a 200 mesh EM Finders Grid (24). The term "at random" requires that every element in a finite population has the same chance of being included in the sample. By employing a table of random numbers, the observer is prevented from introducing his/her own personal preference.

A counting exercise was conducted to examine these and other systematic strategies for grid opening selection, as well as our own proposed random method. All available openings (maximum of 234 opening on each grid) on 2 low density (0.5 fibres per opening) and 2 low-medium density (2.5 fibres per opening) grids were counted. The mean counts estimated using the systematic strategies were compared to the true mean count based on the 234 openings. The results are shown in Table 2. It appears from our data that most of the "non-random" strategies were relatively poor predictors of the true concentration. The cross method (20) gave good estimates but at the expense of counting almost 50 openings. Our random approach was assessed by simulating the selection of 5, 10, 15, 20, 25 and 30 grid openings at random from the 4 grids. Accuracy was excellent for all sample sizes while precision was function of the number of openings and came very close to the expected standard errors based on Normal sampling theory. The computer simulation was also useful in determining the optimal sample size. This is discussed in the next section.

Sampling Strategy

In an environmental sampling program, it is important to consider the variation that may be found within a given sample and between samples. In order to obtain a reasonable estimate of the true concentration, a strategy has to be formulated to address issues such as how many filters should be exposed, how many grids out of each filter should be taken and how many grid openings or number of fibres must be counted. These issues were examined for low fibre density (less than 5 fibres per grid openings) environmental samples (24,25). High inter-filter (between filters) and inter-grid (within filter) variability was found, suggesting that greater accuracy would be achieved by exposing more filters and extracting more grids out of each filter.

The results from the counting trial of all available openings from several low density grids indicated that the count distribution followed the Poisson law (based on Chi Square Goodness of Fit Tests), (Table 3) while the area distribution exhibited clustering effects. The results of counting an entire grid are shown in Figure 4. Each dot represents a fibre and the blackened squares represent damaged openings. Because of this clustering (consistent on all 4 grids), systematic strategies could be very misleading. The computer simulation of random sample selection was also used to determine the optimum number of fields and fibres required for adequate precision. For the low density samples, counting at least 20 grid openings (approximately 50 to 100 fibres total) appeared to be quite adequate.

Identification of Asbestos

In outdoor air, asbestos fibres constitute only a fraction of 1% of all airborne materials. Other mineral and man-made fibres may also be present. To evaluate the associated risk of asbestos in outdoor air, positive identification of asbestos fibres is crucial. We have investigated the following identification methods: Morphology on the Transmission Electron Microscope (TEM), Selected Area Diffraction Patterns (SAED) and microchemical (EDAX) analysis of the fibres.

Elemental analysis by EDAX on various types of standard asbestos (Chrysotile, Amosite, and Crocidolite) has been conducted. We have investigated the positional effect for microchemical analysis of fibres by EDAX. Twenty-four fibres from each type (Chrysotile, Amosite and Crocidolite) randomly selected from UICC Standards were elementally analyzed and sized at 5 locations along the fibre: at each end, at the centre, and at a position equidistant from the centre to each end. A summary by fibre type and elemental ratios (relative to Silicon) is displayed in Table 4. The elemental ratios were analyzed using a repeated measures analysis of covariance - type and position as factors, and overall fibre length and positional diameter as covariates. For all types, fibre location was not significant. Based on the variance estimates, the centre position was found to be the most stable. The length of the fibre also appeared to have no effect.

Since the Silicon levels were highly correlated with diameter and all elemental values were divided by the amount of Silicon, it was felt that the elemental ratios would not be related to the fibre diameter. This was not the case for the Fe/Si ratio in the Crocidolite fibres. A best fit linear regression relating Fe/Si % to the reciprocal diameter had the form:

$$\text{Fe/Si \%} = 82 - 0.89\ (1/\text{Diameter})$$

with $R^2 = 42\%$ and MSE = 7.2 .

Relationship Between PCOM and EM counts

Experimental work is currently underway examining occupational and environmental samples using both PCOM and EM methods. Our tentative strategy involves dividing an exposed 37 mm diameter filter into 8 equal pie-shaped wedges: 2 to be used for PCOM analysis, 4 wedges for direct EM methods, and 2 for use with indirect EM techniques - all will be randomly assigned. Furthermore, we intend to examine at least 1 grid per sample under the optical microscope. To speed up the counting/sizing process, we are employing a microfiche reader at 32X magnification to examine plate film negatives. The entire grid opening is photographed on the EM at 720X magnification. This approach cuts down on the amount of EM time required and reduces observer fatigue while maintaining excellent resolution. We have also developed software on an Apple II+ microcomputer interfaced with a sonic digitizer to measure fibre dimensions directly off the microfiche screen or micrographs.

Biological Relationships

The effects of asbestos air pollution, where the lung is the target organ, can also be evaluated by actual analysis of fibres that are found in the lungs of urban and rural dwellers. Such work involving asbestos in lungs has been done elsewhere (26-28) and should be carried out in Ontario.

CONCLUSION

Since the study's inception, we have established a sample preparation method for EM samples and reviewed various statistical strategies for selecting representative samples. Initial results from our counting experiment suggest that for low density samples, the count distribution is Poisson while the actual area distribution displays clustering. It is imperative that grid openings should be selected for counting at random as suggested in our interim method. Systematic methods of selection appear to underestimate the true mean count.

The work has been successfully initiated in the identification of fibres by microchemical analysis (eg. EDAX) and by selected area diffraction patterns (SAED). Work is in progress in the area of deriving relationships between optical and electron microscope counts so that the extent of health effects of low level asbestos found in outside air could be estimated.

ACKNOWLEDGEMENTS

We are grateful to our research grant monitors, Denis Corr and Paul Roberts of the Ministry of the Environment for the valuable help and advice. Special thanks to Sharon Piedimonte for her patience in typing this manuscript. We are also grateful to the Ontario Ministry of the Environment for funding this research.

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TABLE 1

PROPOSED ENVIRONMENTAL GUIDELINES

1) State of New Mexico (1973)
   - 10 ng/m^3 ambient air regulation

2) Bruckman and Rubino (1975), Connecticut, U.S.
   - 30 ng/m^3 using EM examination
   - based on: a) extrapolation of occupational dose-response
     b) relation 2 f/cm^3(OM) $\equiv$ 10^5 ng/m^3(EM)
   - equivalent to 0.0006 fpcc

3) Sebastien et al., (1977), France
   - 7 ng/m^3 using EM examination
   - based on comparison of outdoor background levels with 21 insulated buildings

4) Ontario MOE, (1981)
   - 0.04 fpcc, fibers > 5μ long by EM count
   - based on 2% of 2 fpcc occupational standard

TABLE 2

MEAN FIBRE COUNTS BY STRATEGY

(NUMBER OF GRID OPENINGS)

GRID NUMBER	TOTAL ●	QUAD 1 ◷	QUAD 2 ◶	QUAD 3 ◵	QUAD 4 ◴	PERIM. ↻	DIAG. ⊗	CROSS ⊕
A-03	2.17 (203)	2.15 (53)	2.00 (49)	2.00 (44)	2.47 (57)	1.66 (38)	1.89 (19)	2.45 (47)
A-02	2.60 (167)	2.75 (44)	2.70 (30)	2.88 (43)	2.16 (50)	2.04 (29)	2.12 (16)	2.85 (46)
L-01	0.53 (214)	0.71 (58)	0.40 (50)	0.54 (52)	0.46 (54)	0.39 (28)	0.35 (20)	0.52 (48)
L-03	0.32 (170)	0.30 (54)	0.26 (35)	0.14 (21)	0.38 (60)	0.31 (48)	0.07 (14)	0.35 (48)

TABLE 3

Grid Counting Statistical Summary

GRID NUMBER	MEAN	VAR.		Number of Fibers 0	1	2	3	4	5	6+	total	χ^2(df)
A-03	2.172	2.124	Obs	28	42	50	48	26	5	4	203	7.6(6)
			Exp	23.1	50.2	54.6	39.5	21.5	9.3	4.0		
A-02	2.599	2.615	Obs	12	37	34	40	22	14	8	167	2.9(6)
			Exp	12.4	32.3	41.9	36.3	23.6	12.3	8.2		
L-01	0.533	0.595	Obs	128	65	16	3	2			214	0.7(3)
			Exp	125.6	66.9	17.0	3.7					
L-03	0.324	0.457	Obs	129	32	5	3	1			170	2.3(2)
			Exp	123.0	39.0		7.2					

TABLE 4 - ELEMENTAL ANALYSIS OF ASBESTOS: Means (Std.Dev.)

Type	Width(μm)	Length(μm)	Si(cps)	Fe/Si %	Mg/Si %	Mn/Si %	Na/Si %
CHRYSOTILE	.05 (.01)	2.9 (1.2)	8.7 (3.1)	7.1 (2.9)	69.8 (5.6)	---	---
AMOSITE	.55 (.36)	13.8 (8.2)	549.8 (378.8)	92.4 (4.7)	10.0 (6.0)	4.4 (1.1)	---
CROCIDOLITE	.23 (.12)	4.3 (4.3)	86.3 (64.1)	77.1 (5.1)	3.2 (0.7)	---	7.4 (0.8)

FIGURE 1

ASBESTOS FIBER DIMENSIONS

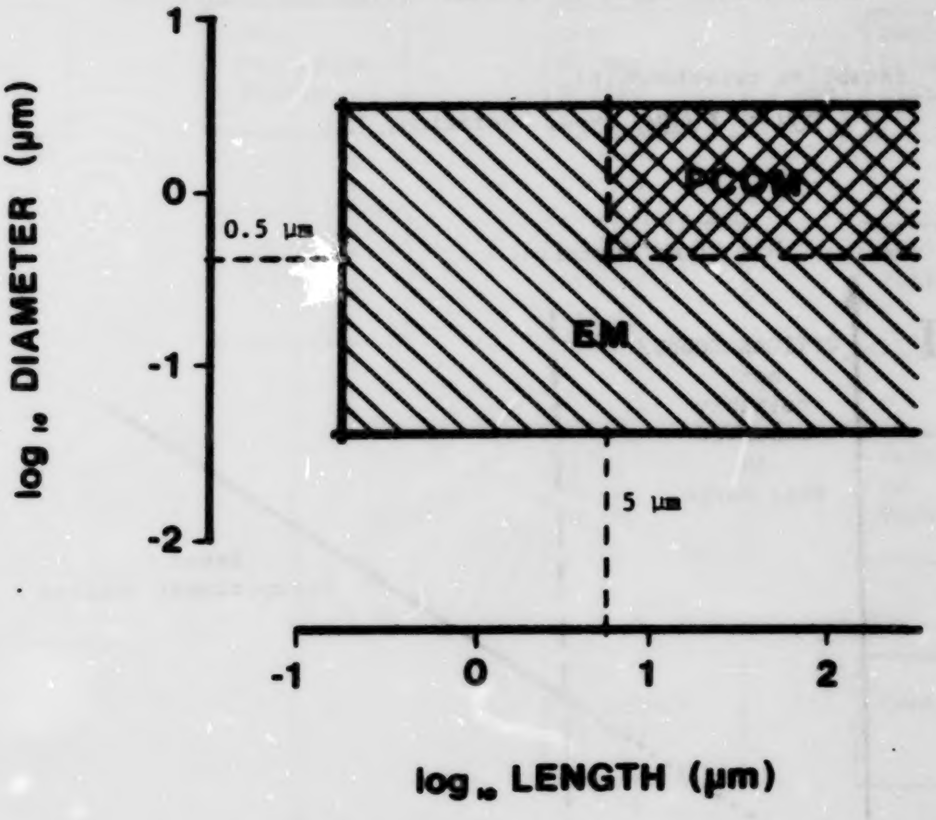


FIGURE 2

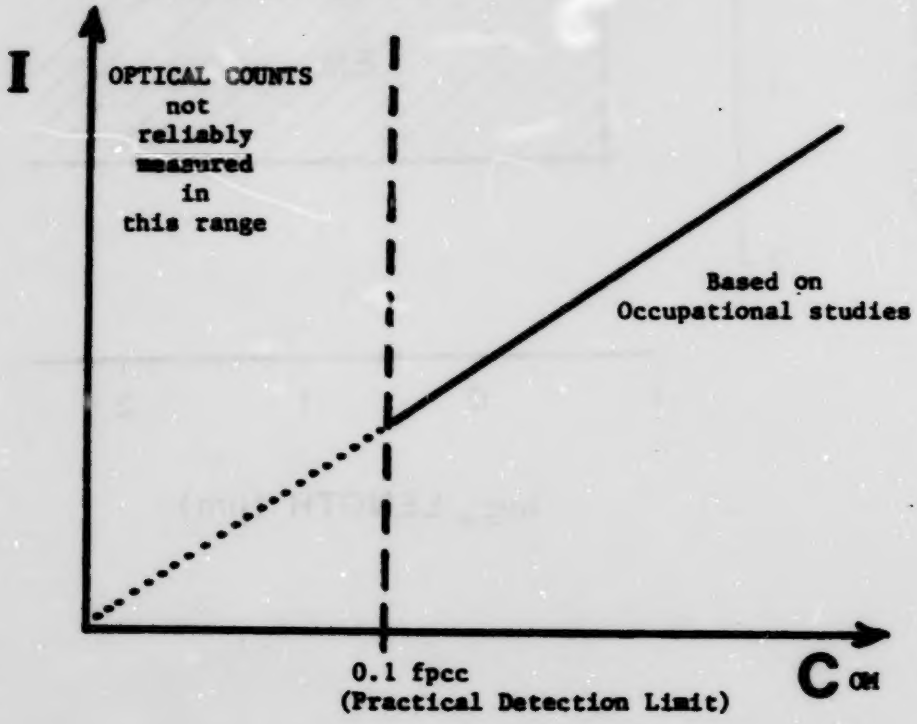
ASBESTOS - linear transformation fits well
- however few data points
- implies NO THRESHOLD LEVEL

Concentration based on Optical Microscope Counts (OM)

Establish relationship:

$C_{OM} = f(C_{EM}$, type, operation, ?)

For fixed duration:



- use $C_{OM} - C_{EM}$ relationship to estimate risk at low levels
- Refinements: 1) allow dose to deteriorate with time (exponential decay)
  2) allow time lag between dose and observed effect.

FIGURE 3

METHODS FOR SAMPLE PREPARATION

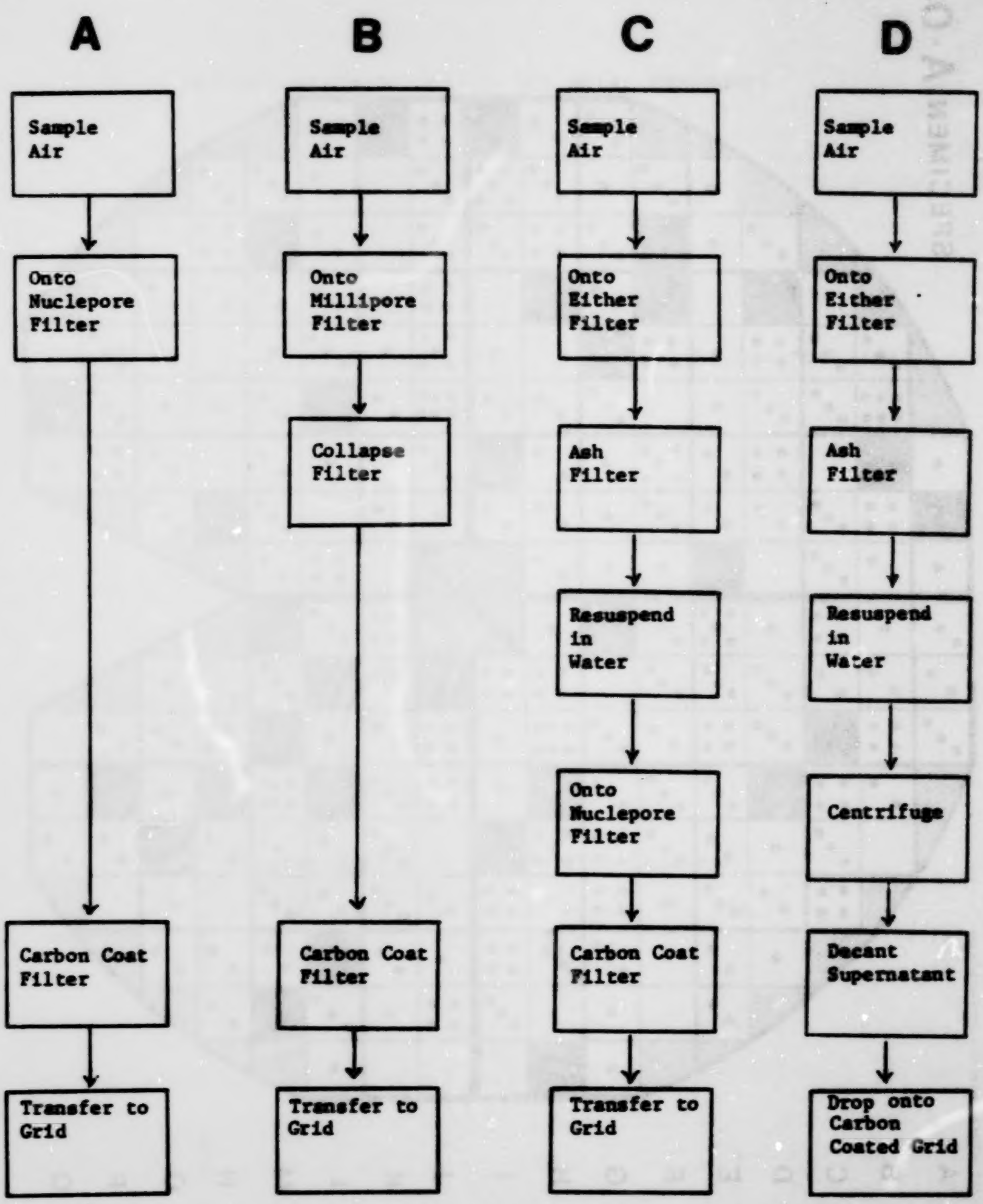
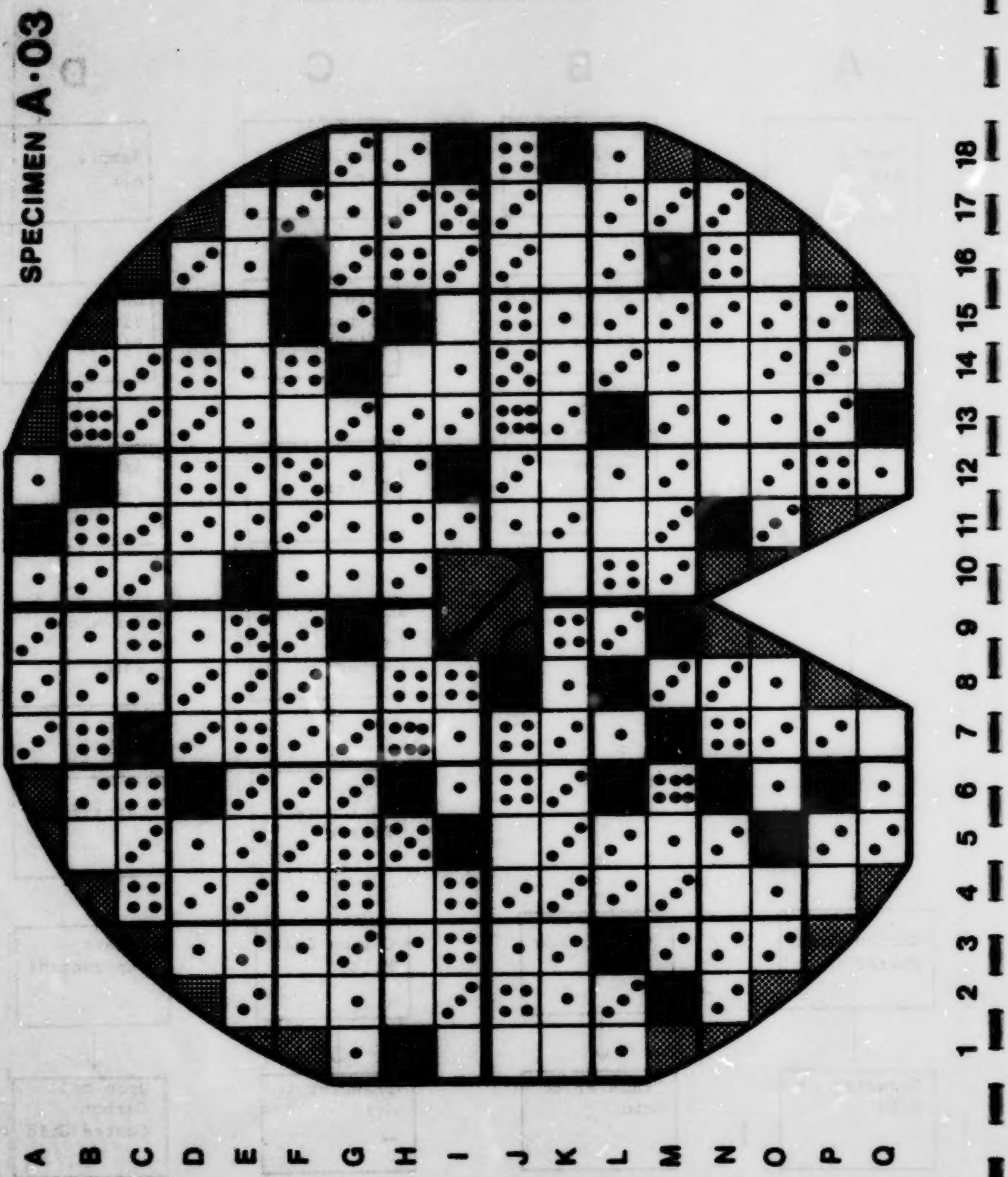

A
B
C
D
Sample Air
Sample Air
Sample Air
Sample Air
Onto Nuclepore Filter
Onto Millipore Filter
Onto Either Filter
Onto Either Filter
Collapse Filter
Ash Filter
Ash Filter
Resuspend in Water
Resuspend in Water
Onto Nuclepore Filter
Centrifuge
Carbon Coat Filter
Carbon Coat Filter
Carbon Coat Filter
Decant Supernatant
Transfer to Grid
Transfer to Grid
Transfer to Grid
Drop onto Carbon Coated Grid

FIGURE 4


SPECIMEN A·03
A
B
C
D
E
F
G
H
I
J
K
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M
N
O
P
Q
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DETERMINATION OF METALS AND METAL COMPOUNDS IN AIR AND RELATED SAMPLES

Jon C. Van Loon, A. Brzezinska, D. Williams, R. Barefoot and
C.M. Nimje

Institute for Environmental Studies
University of Toronto, Toronto M5S 1A1

ABSTRACT:

Work has been concentrated into 2 main areas, a study of Hg compounds in air and water and the direct introduction of air filter samples into an inductively coupled plasma and multitrace element analysis by emission spectrometry.

A. Mercury Compounds in Air and water

A study was made of the gas chromatographic - atomic absorption determination of dimethylmercury and methylmercury chloride in air. Parameters studied include: type of column packing, the influence of the composition of column, transfer line and injection valve surfaces, need for atomic absorption background correction and the efficiency of collection of the compounds. A procedure suitable for analysing building and street air was developed for the determination of dimethylmercury and methylmercury chloride (1). A Tennax column was employed and the temperature program was 150°C to 180°C at a rate at 20°C per minute. Organo mercury results of typical building and urban street air samples will be given. The detection limits for dimethylmercury and methylmercury chloride are 2 and 5 ng respectively. Preliminary results using a microwave plasma in emission as a detector for the gas chromatograph indicate that detection limits may be as much as 10 to 100 time better. A method is being developed for the determination of Hg compounds in water. An extraction of the compounds from samples acidified with HBr into a mixture of benzene and toluene was employed. The latter is carefully evaporated to small volume and an aliquot injected into the gas chromatograph.

B. Direct Analysis of Air Filters

A Perkin-Elmer HGA 2100 was used to produce a vapour of air filters samples. The vapour was carried into the plasma in the sample carrier gas flow. This avoids the need for wet chemical sample treatment and the inherent problems due to losses and contamination. The Procedure involves taking a sample of a glass fiber filter paper using a paper punch. This sample is placed on a graphite platform. The platform is inserted into and centered in the graphite furnace. The furnace is purged with Ar and the flow rate through the furnace is adjusted to the optimum value of 400 ml/min. The sample is volatilized into the furnace. For refractory elements the Ar carrier gas is doped with 0.2% freon (helps volatillize elements as F or Cl compounds). Results have been obtained for Cu, Pb and V. Typical values for Pb and Cu are 4(9) ug/in^2 and 4(3) ug/in^2 respectively (average of several filters). The bracketed values were obtained by wet chemical methods. Values for V will be reported at the seminar.

MERCURY COMPOUNDS IN AIR

SCHROEDER [1] reviewed developments in the measurement of atmospheric mercury. He estimates that 83.5 tons of mercury was released by human activity in 1970. However, he found that few attempts had been made to separate mercury compounds in atmospheric samples. This is in spite of the well-known species dependency of mercury reactions in the environment and in toxicology. Dimethylmercury and methylmercury chloride are the organomercury compounds of interest in air.

BRAMAN and JOHNSON [2] developed a selective absorption type method for the determination of mercury species in air. For example 3% SE-30 on Chromosorb W 45-60 mesh absorbed mercury chloride and Chromosorb W treated with NaOH was used to retain methylmercury chloride. Each fraction was then separately analysed for mercury by DC discharge emission spectrometry. This procedure was presumably lengthy and was used only for the analysis of building air.

It is desirable to have a gas chromatographic method for the determination of organomercury species in air. Such a method should be applicable to samples of outside air. It may also be possible to use such a method, particularly if emission detection is used, to determine the organo species of more than one element simultaneously.

There have been many many reports on the gas chromatography of mercury compounds. However, to the present author's knowledge none exists describing the determination of the important mercury species in outside air samples. In addition there is little data on the optimum chromatographic conditions for the separation of dimethylmercury and methylmercury obtained from any sample type.

There have been 2 comprehensive reviews on the chromatography of organomercurials. FISHBEIN [3] in 1970 reviewed chromatographic and biological aspects of organomercurials, but little on the determination of dimethylmercury was found. In 1978 RODRIQUEZ-VAZQUE [4] reviewed the gas chromatography of organo-mercury II compounds. He makes the important point, which is emphasized in the work reported below, that metal surfaces cause decomposition of organomercurials.

There have been a number of relatively similar gas chromatographic papers starting with the classic paper by WESTOO [5], on the determination of mercury compounds in fish. These involve conversion of the mercury compounds to halides and then extraction into an organic solvent followed by a clean-up step to remove interfering fatty acids. A variety of gas chromatographic column packings have been recommended and often more than one type of column was necessary when dimethylmercury and methylmercury chloride were to be done. The present authors found that there was much confusion in the literature on the type of column to be recommended for determination of dimethylmercury and methylmercury. No gas chromatographic method for the determination of these important compounds in air could be found.

EXPERIMENTAL

Equipment

Chromatography A Fisher 2400 gas chromatograph was employed. It was modified to contain no metal surfaces in contact with the gas stream. A teflon insert was placed in the injector. Glas columns 1 or 2 m long, 5 mm o.d. and 2 mm i.d. were employed. The packing was Tenax or mixed Tenax - DEGS. A Tenax coating using 250 ul of Silyl-8 was also studied. The recommended chromatographic operating conditions are:

Gas (argon) - flow rate 100 ml/min
Injector Temperature - 200°C
Column Temperature Program - 150°-180°C/min.
A Teflon tube transfer line heated to
140°-180°C was employed

Atomic Absorption Detector. A Perkin-Elmer 305B atomic absorption unit equipped with a deuterium arc background corrector was used. The radiation source was an mercury hollow cathode lamp. An Omniscribe (Houston Instruments) single pen chart recorder was employed at a chart speed of 0.5 cm.min. The wavelength and slit widths were 253.6 nm and 0.7 nm respectively.

A quartz "T" tube atomizer was employed. This consisted of a quartz capillary (5 mm o.d. and 1 mm i.d.) pyrolyser (at 900°C) terminated by an open ended unheated 15 cm long by 1-15 cm i.d. quartz tube absorption cell (Fig 1). The addition of CuO to the pyrolyser as recommended by others was found to have little useful effect in this work.

Reagents

Dimethylmercury (Alfa Inorganics). A stock solution was prepared by weighing 0.05 ml into a 50 ml volumetric flask and diluting it with benzene. This was kept in a refrigerator at about 4°C. Working solutions were prepared fresh daily by dilution in benzene.

Methylmercury chloride (Alfa Inorganics). A sample weighing 100 mg was dissolved in 50 ml of benzene. The stock solution was kept in a refrigerator at about 4°C. Working solutions were prepared fresh daily in benzene.

Recommended Procedure

Air samples were collected on a short piece 14 x 0.8 cm length of glass chromatographic column packed with 20% Tenax and 80% 1 mm glass chips. Pumping speed was 2 l/min. Air was first drawn through a Milipore membrane filter of 0.45 u pore size. After the air sample had been collected on the short column, the latter was connected to an argon supply and the mercury species desorbed by heating the column to about 200°C for 0.5 hour. An argon flow rate of 10 cm/min was used. The mercury compounds were collected in 0.1 ml of benzene in a micro impinger. The impinger solution was cooled in an ice salt water bath. [temp. about 6°C]. Appropriate aliquots of benzene solutions (10 or 20 ul) containing standards or sample were injected into the gas chromatograph. A temperature programs of 150°C to 180°C at 20°C per min. was used. A 1 m column was used with a carrier gas flow rate of 100 ml/min. Retention times for dimethylmercury and methylmercury chloride were 0.3 min and 1.8 min. respectively.

RESULTS AND DISCUSSION

Study of Column Packings

A variety of column lengths and packings were studied: Results are summarized in Table (1).

(1) 15% DEGS on Chromosorb W 80/100 mesh - 2 m length;

(2) 15% DEGS on Chromosorb W 80/100 mesh (1.5 m length); and Tenax 80/100 (0.5 m length)

(3) 15% DEGS on Chromosorb W 80/100 mesh (1 m length) and Tenax 80/100 mesh (1 m length);

(4) 15% DEGS on Chromosorb W 80/100 mesh (o.5 m length) and Tenax 80/100 mes
length);

(5) Tenax 80/100 mesh (2 m length);

(6) Tenax 80/100 mesh treated with Silyl 8 (2 m length);

(7) Tenax 80/100 mesh (1 m length);

Table 1. Retention Times of Mercury Compounds

Column	Length m	Temperature conditions	Retention time (min)		
			Hg Vap	$(CH_3)_2Hg$	CH_3HgCl
1. Tenax 80-100 mesh	1	150-180° 20°/min	-	0.3	1.8
		80-180° 20°/min	0.9-0.6	2	6
2. Tenax 80-100 mesh	2	80-180° 20°/min	0.5-0.	3.8-4	10-11
3. 75% Tenax 25%(15% DEGS) on Chromosorb W 80-100 mesh	1.5 0.5	70-100 4°/min for 15 minutes 100-180° 20°/min	1	4	16

DEGS. A packing of chromosorb W coated with 15% DEGS [6] is usually used for methylmercury determinations. In our work the best resolution between dimethylmercury and methylmercury chloride was obtained using temperature programming of 60-140°C at 20°/min e.g. (Fig.2). Under these conditions it was impossible to separate the mercury vapour peak, if present, from dimethylmercury. Changing of the carrier gas flow rate did not improve the situation. This packing cannot be used when mercury vapour is present with dimethylmercіry.

The sensitivities amounts give 0.044 absorbance; for this column are 8 and 40 ng of mercury in dimethylmercury and in methylmercury respectively.

TENAX and DEGS. When the column contained 50% DEGS on Chromosorb W and 50% of Tenax good separation between dimethylmercury and methylmercury was obtained under isothermal conditions (175°C) e.g. Fig.3. The separation of mercury vapor, if present, from dimethylmercury was difficult even at low temperatures. Sensitivities for the mercury compounds are similar to those obtained on a column containing only DEGS. The repeatability of the signal due to methylmercury chloride was poor.

As the amount of Tenax is increased the separation of mercury vapour, if present, and the two organo-mercury species improve. With a complex two step temperature programming (a) from 70 to 100°C with a rate of 4°/min and (b) from 100 to 180°C with a rate of 20°/min the resolution was very good [Fig. 4]. Retention times obtained with 15% DEGS (25%) - Tenax (75%) column are given in Table 1.

The amounts of mercury corresponding to 0.044 absorbance were 4, 5-6 ng and 33-35 ng for mercury vapour, dimethylmercury and methylmercury respectively. It was difficult to obtain good repeatability for methylmercury chloride.

TENAX. The separation of mercury vapour, when present, from the two organo-mercury species is best when a 100% Tenax packing was used with a 2 m column e.g. Fig. 5. [A simple one programming from 80-240°C with a rate of 20°C/min was used for Fig. 5]. The retention time for mercury vapor is the same as for the mixed DEGS-Tenax columns and for dimethylmercury and methylmercury chloride is greater with increasing amounts of Tenax.

The retuention time of methylmercury on Tenax columns was quite long. Because the separation of the mercury species was good it was found that a column as short as 1 m could in practice be used. (Fig. 6). The separation between dimethylmercury and methylmercury chloride is suitable for routine work using a programmed temperature from 150 to 180°C at 20°C/min. The retention time obtained with 1 and 2 m Tenax Columns are given in Table 1. An absorbance signal of 0.044 was obtained for 3.5 ng of Hg in dimethylmercury and 13-14 ng of Hg in methylmercurychloride.

After using a Tenax column for 2-3 weeks [the 2 m column is worse] some empty spaces (dead volume) are observed in the packing and the methylmercury chloride peak becomes irregular and repeatability decreases. To eliminate this problem the column was treated with Silyl-8 [Chromatograph Specialities Brockville Canada]. Ten 25 ul injections at 150°C were used. This eliminated the irregularities int he methylmercury peak. The sensitivities remained roughly the same.

Effect of Benzene

The chromatogram given in Figure 7 shows the methylmercury and benzene peak obtained at 180°C. When programming is used the benzene peak is very broad but is usually corrected by the deuterium arc background corrector. At higher temperatures benzene gives a very large absorbance which is not correctable.

A few times when even using the recommended procedure, a small benzene peak was obtained. This may have been due to the poor alignment of the atomizer in the optical beam or failure to have the deuterium arc light beam and the hollow cathode lamp light beam filling the same optical aperture.

Nature of Chromatographic Surfaces

In much of the early work in this study there was evidence of losses or decomposition of the mercury compounds. This was in spite of a concerted effort to prevent leaks and not to have exposed metal surfaces [in a similar study with Pb[7] some metal surfaces were found to decompose tetraalkyllead compounds]. It became evident that on an all glass system [with a minimum of Teflon connectors] optimized the results. Such a system, though essential, is extremely difficult to use. When Teflon is used the maximum column temperature should be reduced to 180°C. The importance of using such a system cannot be over emphasized.

Collection Efficiency

A column packed as for collection of mercury species from the air was placed in the gas chromatograph. Gas was then passed through the column for 48 hours. The compounds were chromatographed using the conditions of the proposed procedure. Approximately 100% recovery was obtained for methylmercury chloride. Recovery for dimethylmercury was 98% as long as the collection was done at a temperature of 18°C or below. If the collection is to be done above this temperature then the collection tube should be cooled in a cold water bath during the collection-period. Peak areas were used in the above test because the chromatographic peaks (especially dimethylmercury were broader than those of the standards).

Repeatability

A number of injections of the two compounds (100 ng of mercury in methylmercury chloride and 8 ng of mercury in dimethylmercury were made and the chromatography done using the recommended conditions. The coefficients of variation obtained were 7% for methylmercury chloride and 6% for dimethylmercury.

Determination of Organo Mercury Species in Air.

During air sampling air was passed through the flow-meter only momentarily to test the flow rate. The average flow through the column trap was 2 l/min. In our work on street air it was necessary to sample for 10-30 hours. After the sampling was finished the column was connected to the argon tank. The trap was heated using a heating tape to about 200°C for 0.5 hour and the compounds collected into 0.1 ml of benzene in the impinger cooled in ice. A slow flow of Ar (10ml/min) removed the mercury compounds from Tenax into the benzene. All the organic mercury compounds are retained by Tenax but only a part of the elemental mercury. For this reason mercury vapour was not included in the procedure. Figure 8a shows a typical chromatogram for a 20 ul injection of benzene, from collection of a real air sample, equivalent to 2 ng of Hg in dimethylmercury and 20 ng of Hg in methylmercury chloride. Table [2] is a compilation of results obtained during different days and for different lengths of collection. Detection limits are 2 ng and 5 ng for dimethylmercury and methylmercury chloride respectively.

Table 2 - Concentration of Mercury Species in Air

I. Room 322 Mining Building			
Sample	Volume of air (m^3)	Dimethyl mercury ($ngHg/m^3$)	Methylmercury chloride ($ngHg/m^3$)
1	15	0.5	30
2	16	tr	18
3	10	tr	14
4	10	tr	3
5	18	0.6	5
6	25	0.2	3
7	12	tr	3
8	12	1.3	2
II. Outside at 170 College St.			
1	30	0.4	tr
2	25	0.4	tr

CONCLUSIONS

A relatively simple method of the determination of organic mercury compounds by gas chromatography-atomic absorption spectrometry was described. Using a Tenax 2 m long column and programming from 80 to 180°C at 20°/min the separation of mercury vapour from dimethylmercury and methylmercury chloride is possible. In this case dimethylmercury peak appears at 120°C and the sensitivity of this compound is lower than at higher column temperatures. Therefore a short programming step from 150 to 180°C at 20°/min is recommended when the analysis is limited to organic mercury compounds. In both cases the background correction is important.

The direct connection of mercury collection trap to the gas chromatography resulted in broadened peak of dimethylmercury. This approach may be applicable with further research.

The work is now being continued to show the applicability of microwave plasma emission spectrometry as a detector for gas chromatography using the above methods.

DETERMINATION OF METHYLMERCURY IN WATER

Mercury levels in most natural waters are expected to be below 1 ug/l. Thus organomercury (most of which is expected to be methylmercury) will be some fraction of this amount. It is essential therefore to develop a very sensitive method for the determination of methylmercury in water. A solvent extraction procedure was developed.

EXPERIMENTAL

Equipment

Chromatographic and atomic absorption equipment is similar to that used for the determination of Hg compounds in air. A water bath kept at 85°C is used to evaporate solvents.

Chromatographic Conditions are:

Column	Glass 1.5 m x 5 mm o.d. packed with Tenax 80-100 mesh

Column temperature 180°C

Injection temperature 200°C

Transfer line - Teflon tubing temperature 160-180°C

Reagents

Methylmercury chloride (Alfa Inorganics)

A sample weighing 100 mg was dissolved in 50 ml of benzene or toluene. The solution was kept at 4°C. Working solutions were prepared fresh daily in benzene or toluene. Baker analysed benzene and toluene were used.

Recommended Procedure

Samples were collected in acid washed (glass) bottles. They were stored at 40°C until extracted. (It is highly recommended where possible to do the extraction in the field.) Prior to analysis samples were allowed to warm to room temperature. One litre samples were used for extraction. Two g of KBr was added to the water in the sample bottle and the solution agitated to dissolve this material. (If there is difficulty in obtaining an emmulsion free separation 0.5 ml of Hf should be added at this stage.) A 5ml portion of a 3:1 mixture of benzene and toluene was added. The sample was vigorously shaken for 60 s and then allowed to settle for 20 m. All but 50 ml of the water layer was syphoned off and discarded. The reamining solutions were transfered to a 100 ml separatory flask and the layers carefully separated.

Standard solutions were extracted in an identical manner to the samples. One liter methylmercury standards containing 50, 100, 200 and 250 ng Hg were prepared and reagent blank was also run. Four ml alignots of the organic extracts were placed in separate small glass vial impinger tubes. The solvent was evapourated to 0.1 ml in a water bath kept at 85°C. The final stage of the evaporation was watched very carefully to avoid haivng the evaporation go to dryness.

Twenty ul alignots were injected into the gas chromatograph. Chromatographic conditions as listed above were employed.

RESULTS AND DISCUSSION

Maximum Gas Chromatographic Injection Volume

It is desirable to use as large a portion of the 100 ul extract as possible in the gas chromatograph. Fifty and 100 ng of Hg as CH_3HgCl in different volumes of benzene (from 1 to 50 ul) were injected into the gas chromatograph. The recommended chromatographic conditions were employed. Results show that above 20 ul there is a slight decrease in sensitivity.

Effect of Salt Contents on the Extraction

A variety of salts were tested to determine their effect on the extraction efficiency. These were NaCl, KCl, KF, KBr and KI. Of these KBr showed a 30% and 100% improvement over chloride and iodide salts respectively.

The effect of different salt content on the extraction efficiency was investigated. The results are given in Table (3)

Table 3. Effects of NaBr on Extraction Efficiency (%)

Hg(in CH_3HgCl) ug/l	Salt Content (%) 0	0.1	0.2	0.5	1.0	2.0	5.0	10.0
10	88	94	97	96	83	88	78	62
20	90	43	98	97	92	87	80	63
30	92	95	98	97	93	86	79	60
40	91	94	97	96	93	85	80	60
50	92	96	98	97	92	86	80	60

As can be seen there is a rather narrow range of salt concentrations giving quantative recoveries. A concentration of 0.2% was chosen for the proposed procedure.

Effect of Sample pH on The Extraction

The pH of the sample solution was varied from 1 to 10 using HNO_3 and NH_3. The extraction procedure was then employed for each solution. No significant difference in extraction efficiency was noted over this pH range.

Effect of Shaking Time on the Extraction

A relatively large volume of water compared to solvent volume must be employed. For this reason it is important to investigate the length of time of shaking reguired to obtain a maximum recovery of the CH_3HgCl during the extraction.

Various shaking times from 30 s to 30 m were employed on solutions prepared as indicated in the proposed procedure. Suprisingly 20 to 60 s were found sufficient to give maximum recoveries.

Composition and Volume of Solvent

It was necessary to evaporate the solvent prior to injection into the gas chrometograph. Loss of Hg is, of course, possible during this operation. Losses of up to 40% were obtained using benzene. Thus various volumes of benzene mixed with toluene were investigated to seek an improved recovery. The proposed procedure was employed and the results are given in Table (4)

Table 4. Effect of Solvent Composition on Recovery of CH_3HgCl

Percentage of Tolnene	Recovery %	Evaporation Time hr.
0	66	3.5
5	72	4.2
10	88	4.5
20	96	4.8
25	98	5.0
50	90	5.5

The minimum volume of solvents which can be used for 1 l of water was found to be 5 ml. When the volume of water was increased the volume of solvent needed must be increased proportionately. A 3:1 mixture of benzene to toluene was chosen.

Analysis of Water by the Proposed Procedure

A number of river water samples were analysed by the proposed procedure. Results are given in Table (5).

Table 5. Determination of CH_3HgCl in Waters (ng/l).

Water	Sample	Result	Coefficient of variation
Humber	1	25	5
	2	25	5
	3	30	5
Creek (Toronto)	1	30	10
Creek (Mississauga)	1	45	10

These are the first results for methylmercury chloride in Toronto waters.

Because of the very low levels of CH_3HgCl in rain and lakes this compound has not been detected in such samples at this time of writing. However, as will be seen in the next section our work with the microwave plasma as a gas chromatography detector shows that better detection by at least 2 orders of magnetude have been achieved. Thus we will be doing the determination of CH_3HgCl in rain and lake water soon.

MICROWAVE PLASMA EMISSION DETECTOR FOR GAS CHROMATOGRAPHY

Work reported in the previous two sections of this report indicates that better detection limits are needed. The levels of mercury compounds in outside air and water are so low that work with the atomic absorption detector only allows analysis of relatively polluted samples. Thus we are developing a microwave plasma emission detector for use with the gas chromatography.

EXPERIMENTAL

Equipment

The microwave plasma was generated with a Microtron 200 Mark II 0-200 W power supply. The cavity was homemade and is our modification of the Beenakker (8) design (TM_{010}). Radiation was focussed by means of a 50 mm focal length by 25 mm diameter fused SiO_2 lens on the slit of a model H-20 Instruments S.A. Inc. monochromator. This monochrometer has 0.1 mm slits and a bandpass of 0.4 nm. The 253.7 nm Hg line was employed. A solar blind R166 (Hanamatsu TV) photomultiplier was used. Gas chromatography was similar to that reported in Section (1).

Reagents

These were similar to those used in Section (1).

Procedure

This was similar to the procedure in Section (1).

RESULTS AND DISCUSSION

Optimization of Experimental Conditions

The retention time for CH_3HgCl was 3.5 minutes. The system was optimized for the following parameters.

(1) flow rate
(2) forward microwave power
(3) spatial variations

The flow rate will effect the plasma and the chromatography system. The signal is optimal for a flow rate of a 130 $ml.min^{-1}$ Therefore the emission signal is maximum at a flow rate of a 130 $ml\ min^{-1}$.

The efficiency of the column, as measured by the number of theoretical plates, was calculated using the following squation:

$$N = 5.54 \left(\frac{tr}{W^1/_2}\right)^2$$

where tr = retention time and $W^1/_2$ equals the peak width at half height. The column efficiency is at a maximum at low flow rates. However, the efficiency at the flow rate where the emission maximum occurs is not significantly lower than the maximum efficiency.

The microwave power is optimized at a level of 70 watts. The system was previously found to be at an optimum in terms of spatial variations, when the centre of the plasma was focussed on the entrance slit.

Detection Limits

Although the detection limit has not been experimentally established, it can be estimated to be between 10 and 30 pg. This detection limit is much better than the detection limit for G.G./A.A.S. which is 5 ng for methylmercuric chloride.

Dynamic Range

Linearity of response has been observed over a limited range (0-2 ng). However it is expected that the dynamic range will extend over several orders of magnitude.

Reproducibility

The reproducibility for CH_3HgCl is about ±3% for a 1.25 ng sample (peak height).

Conclusion

The G.G./M.I.P. has great potential as a detector for CH_3HgCl in air and water samples. It has good sensitivity and high selectivity.

TRACE ELEMENTS IN AIR FILTERS BY DIRECT INJECTION OF FILTER AEROSOL INTO ICP

An air filter sample is analysed by placing a punched out circle of the Filter into a graphite furnace which is connected to the base of the ICP torch. The furnace is rapidly heated and the aerosol thus produced is swept into the plasma.

EXPERIMENTAL

Equipment

An Applied Research Laboratories 34000 Quantometer which is supplied with a PDP 11 - computer (Digital) was used.

A Perkin Elmer HGA 2100 was used to produce the sample aerosol (vapour). The furnace housing was very easily adaptable to interface with the torch of the plasma through a Quartz tube friction fitted to a graphite ring in one end of the furnace.

The two components are interfaced with a J. tube. The complete interfacing module consists of

(1) "j"-Quartz transfer tube with valve and side arm.

(2) Pressure reducing system (concentric Meinhard nebulizer).

(3) Flowmeters with high precision valve controls.

A photograph of the equipment is shown in Fig.9. A schematic of the system is given in Fig.10.

ICP Operating Conditions are:

Incident power	1300 W
Reflected power	<10 W
Coolant gas pressure	50 PSI
Plasma gas pressure	35 PSI
Carrier gas flow rate	0.5 Litres min^{-1}
Sample aerosol flow rate	0.4 Litres min^{-1}

Furnace operating conditions are:

Drying time	0
Ashing time	20 sec.
Ramp.	10 sec.
Temp.	200°C - Pb
	650°C - Cu
Atomization/volatilization time	8 Cu
Ramp.	0
Temp.	>2800°C

Reagents

All reagents were Reagent Grade ACS. Stock metal standards were prepared from spec pure metals dissolved in a minimum amount of nitric acid. Part per billion working solutions were prepared fresh daily.

Procedure

A punch is used to obtain a sample of the glass fibre filter paper. The sample is then pressed into the slot on a graphite boat. The aluminium cap is removed and the boat slid into the furnace tube. The boat is centered using a centering tool. The bypass valve is then opened and the furnace purged with Argon. The flow rate is set to the operating level of 400 ml Min^{-1}. The furnace program is then actuated. The peaks are recorded on the chart recorder. Calibration standards are prepared by pipetting ul volumes of standard solutions on a punched out piece of blank glass fibre filter paper. Standards are run in the same way as the samples.

RESULTS AND DISCUSSION

Oxygen Problem

The argon plasma is sensitive to the presence of oxygen in the system and will extinguish easily if a sudden surge of air or argon/air mixture is introduced into its central channel. A bypass valve on the transfer tube is designed to iosolate the furnace from the torch during insertion of a solid sample into the furnace.

Argon sample uptake flow rate

There are 3 gas flows to the plasma torch. The sample aerosol (vapour) from the furnace is injected into the central axial channel. The optimum flow for this is between 0.7-1.0 live min^{-1}. In order to maintain a stabilized plasma, this argon flow is split up into two fractions, one fraction is fed directly and continuously into the central channel and the second fraction is routed through the furnace. Experiments have been performed to optimize this splitting for both plasma stability and signal response.

Results

Air filters were run by the proposed procedure for Pb and Cu and the results given in Table (6).

Table 6. Results: (ICP) Values for Pb, Cu and V in Air Filters

SAMPLE #	SOLID SAMPLING $ug.\ m^{-3}$			CONVENTIONAL DIGESTION $ug.\ m^{-3}$		
	Pb	V	Cu	Pb	V	Cu
1	1.3	3.3	0.8	1.6	5.7	0.8
2	2.6	9.6	1.6	4.3	12.0	1.2
3	2.4	4.7	0.8	3.0	4.8	0.4
4	3.5	4.5	1.2	4.9	5.5	1.2
5	4.3	7.3	2.4	8.3	8.8	1.6

The results were compared with those obtained by a conventional digetion. Acceptable agreement was obtained for Cu. The results at this stage are not as good for Pb and V.

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LIST OF FIGURES

Fig.1 Heated teflon transfer line, quartz decomposition tube and absorption cell.

Fig.2 Chromatogram of mercury compounds separated on a 2 m column packed with 15% DEGS on Chromosorb W 80-100 mesh:
Injector temperature - 150°C
Column temperature - 60-140°C programmed at 20°/min
55 ng of mercury in dimethylmercury
750 ng of mercury in methylmercury chloride.

Fig.3 Chromatogram of mercury compounds separated on a 2 m column packed with 15% DEGS on Chromosorb W 80-100 mesh (50%) and Tenax 80-100 mesh (50%).
Injector temperature - 200°C
Column temperature - 160°C
40 ng of mercury in dimethylmercury
300 ng of mercury in methylmercury chloride.

Fig.4 Chromatogram of mercury compounds separated on a 2 m column packed with 15% DEGS on Chromosorb W 80-100 mesh (25%) and Tenax 80-100 mesh (75%).
Injector temperature - 200°C
Column temperature: 70-100°C programmed at 4°/min and from 100-180°C programmed at 20°/min
20 ng of mercury vapour
40 ng of mercury in dimethylmercury
500 ng of mercury in methylmercury chloride.

Fig.5 Chromatogram of mercury compounds separated on a 2 m column packed with Tenax 80-100 mesh.
Injector temperature - 200°C
Column temperature - 80-240°C programmed at 20°/min
20 ng of mercury vapour
10 ng of mercury in dimethylmercury
400 ng of mercury in methylmercury chloride.

Fig.6 Chromatogram of mercury compounds separated on a 1 m column packed with Tenax 80-100 mesh.
Injector temperature - 200°C
Column temperature - 80-240°C programmed at 20°/min
400 ng of mercury in dimethylmercury
300 ng of mercury in methylmercury chloride.

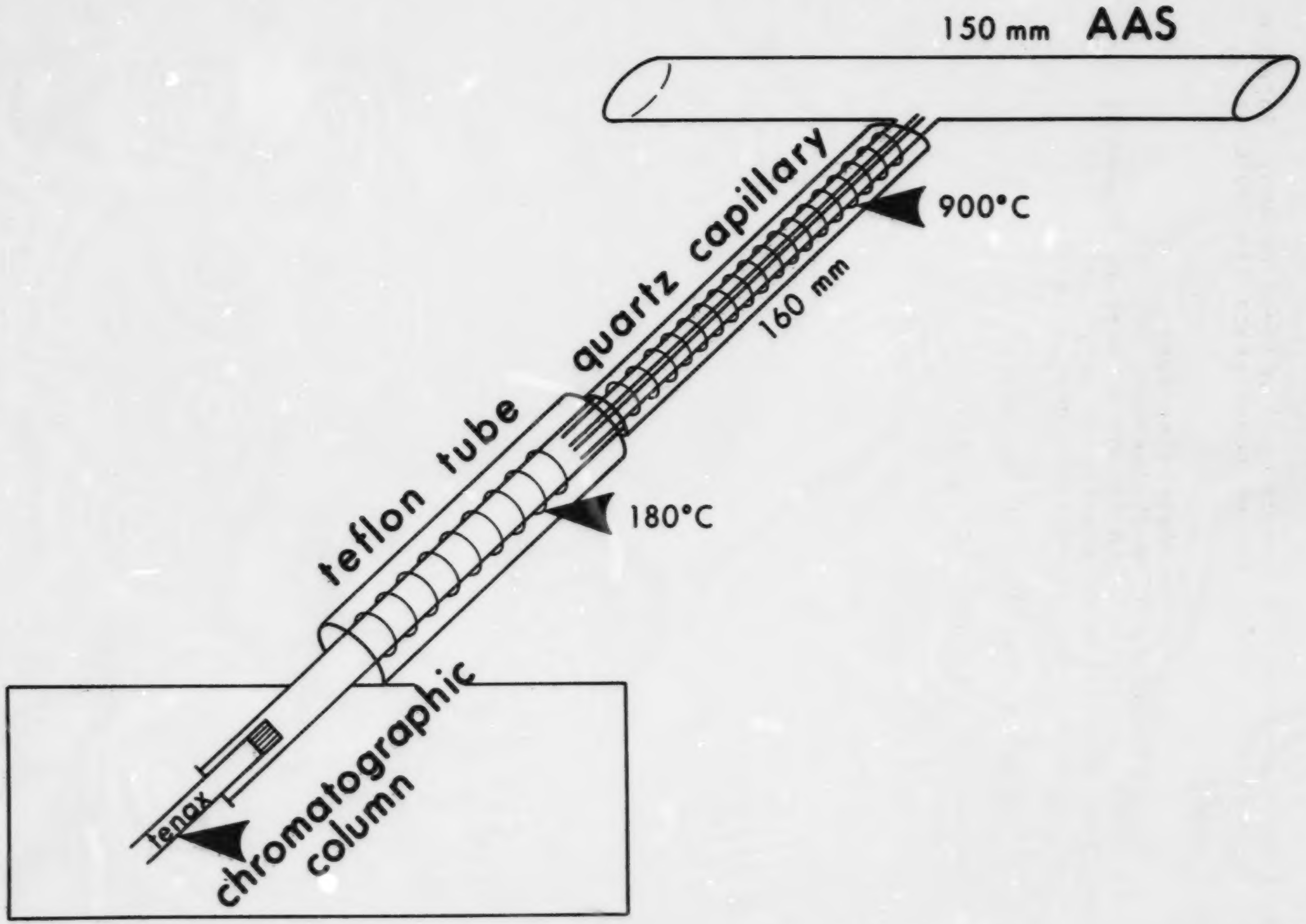
Fig.7 Effect of benzene. Column - 1 m packed with Tenax 80-100 mesh.
A-200 ng of mercury in methylmercury chloride at constant temperature (180°C)
B-200 ng of mercury in methylmercury chloride at programmed temperature (80-100°C at 20°C/min).

Fig.8 Chromatogram of air sample on 1 m Tenax column. Injector temperature -200°C, column temperature 150-180°C at 20°C/min.

A.3.2 m^3 of air
  2 ng of mercury in dimethylmercury
 20 ng of mercury in methylmercury chloride
B.3.2 m^3 of air with additions of mercury standards
  5 ng of mercury in dimethylmercury and
 25 ng of mercury in methylmercury chloride

Fig.9 Photograph of Furnace ICP Equipment.

Fig.10 Schematic of Furnace ICP system.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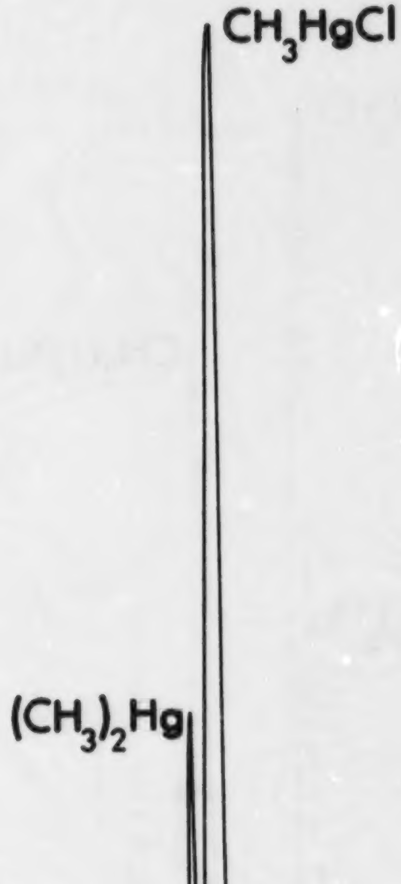
AAS
quartz capillary
900°C
160 mm
teflon tube
180°C
chromatographic
column
tenax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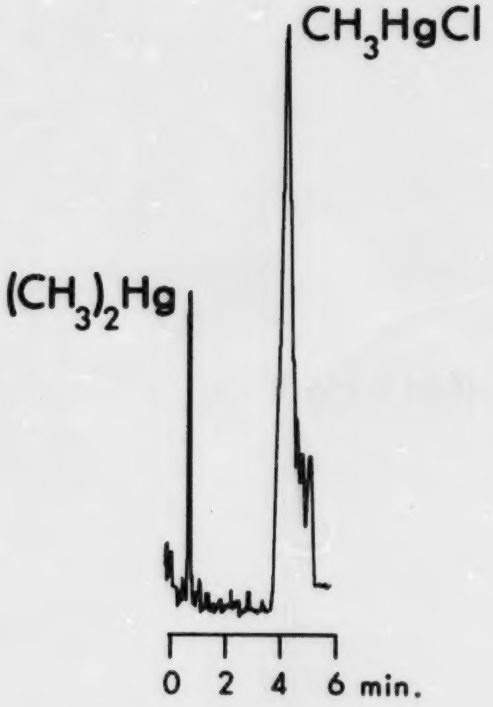
0
2
4 min.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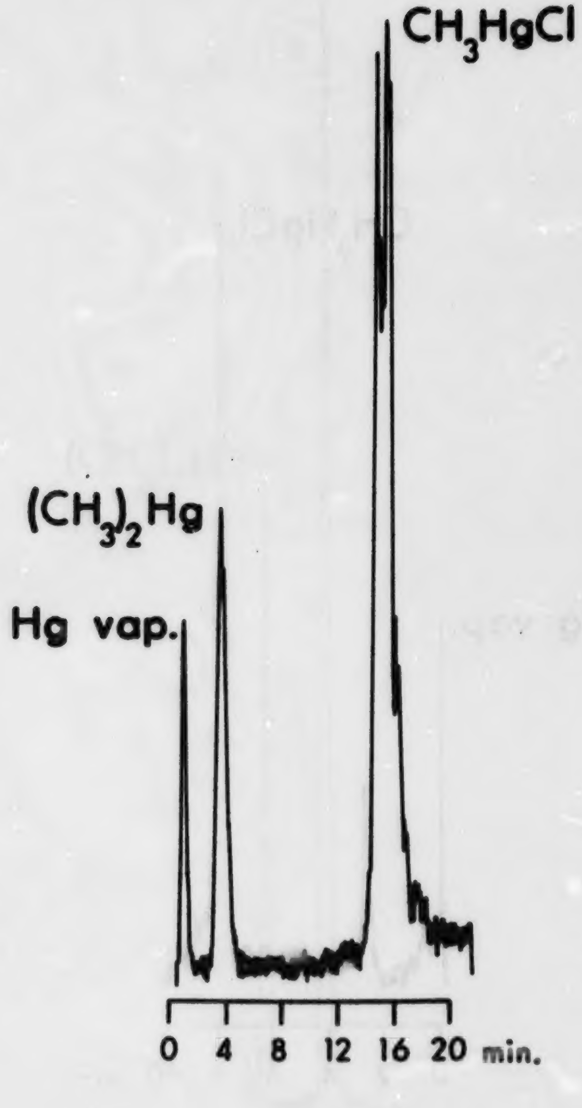
(CH$_3$)$_2$Hg
0
2
4
6 min.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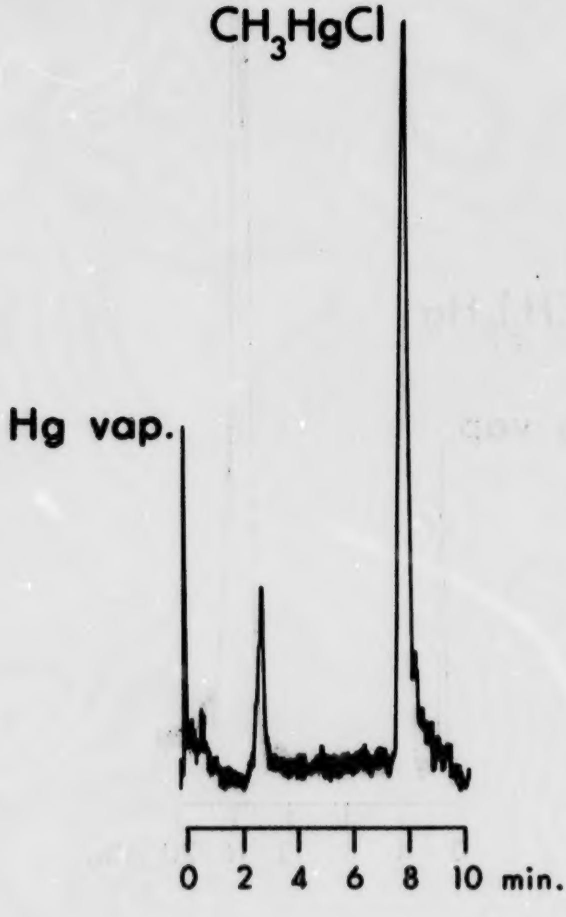
(CH$_3$)$_2$Hg
Hg vap.
0
4
8
12
16
20
min.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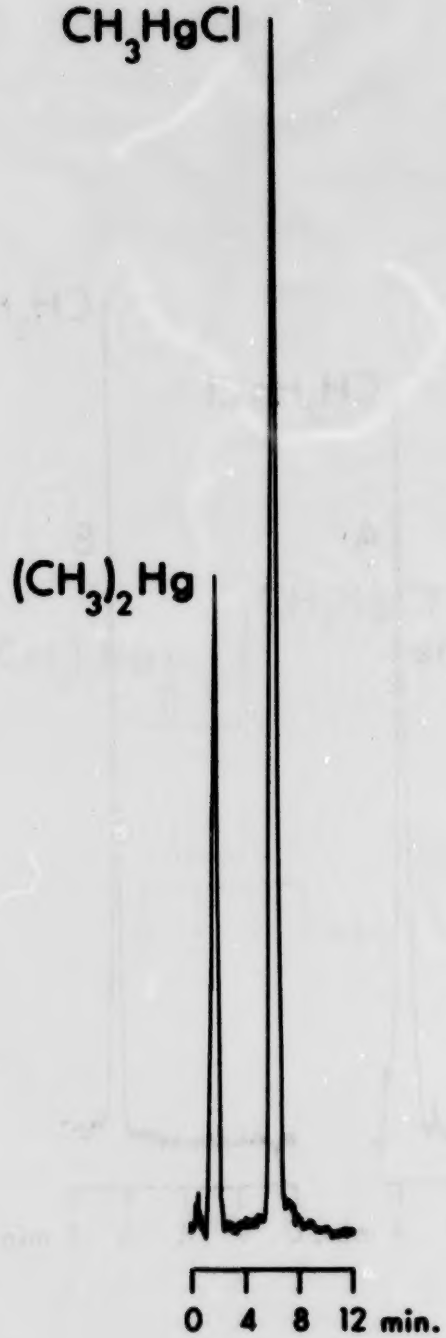
Hg vap.
0
2
4
6
8
10
min.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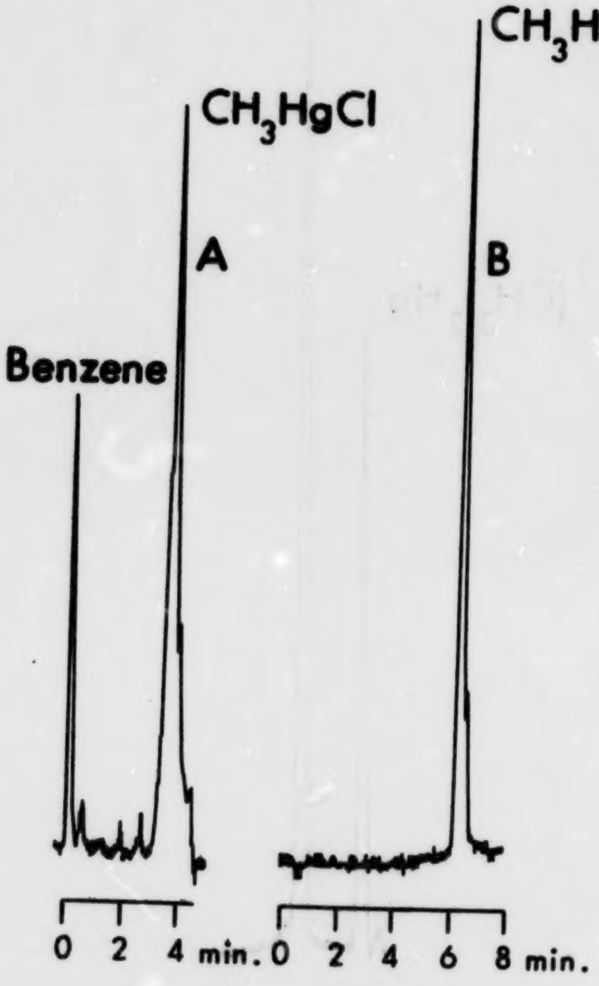
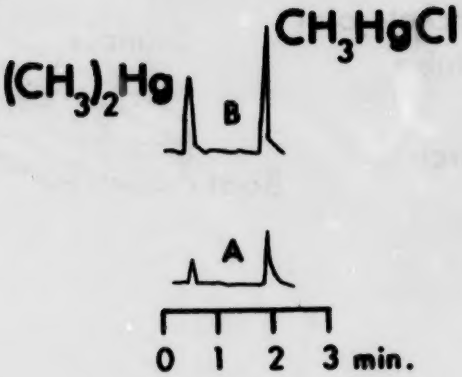
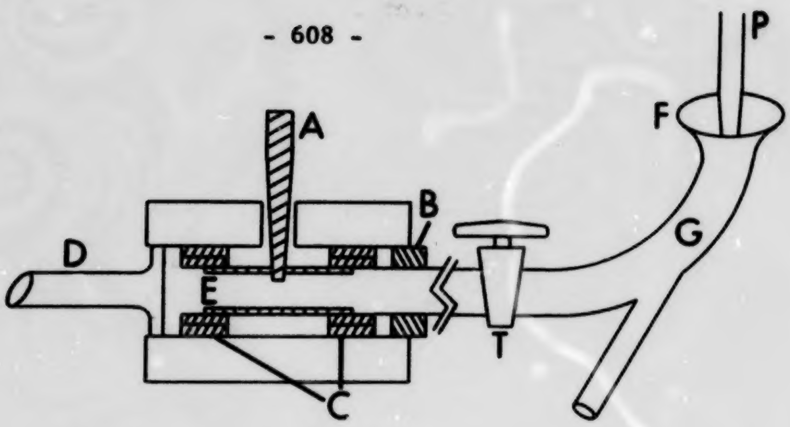
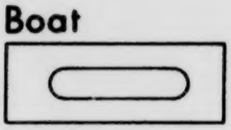
4
8
12 min.

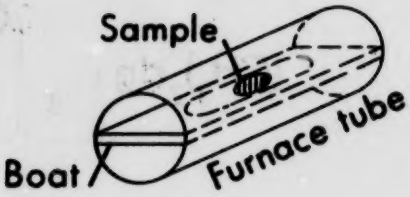

CH$_3$HgCl
A
Benzene
0 2 4 min.
CH$_3$HgCl
B
0 2 4 6 8 min.

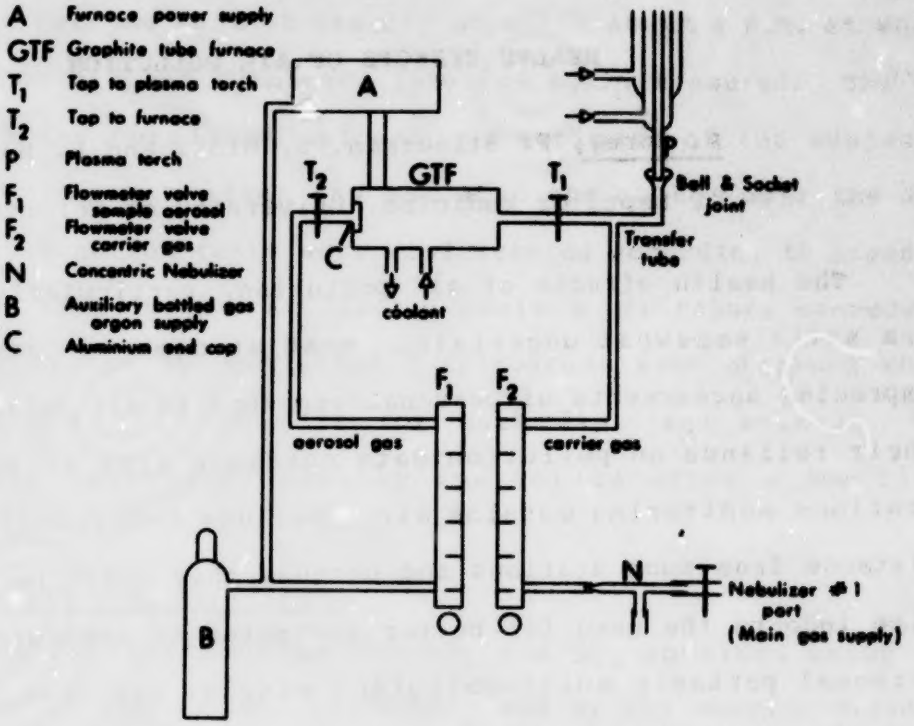

CH₃HgCl
(CH₃)₂Hg
B
A
0 1 2 3 min.



A	Graphite plug
B	Graphite bushing
C	Graphite cones
D	Aluminium end cap
E	Furnace tube
F	Ball & Socket joint
G	Transfer tube
T	Tap
P	Plasma torch




Sample
Boat
Furnace tube


A Furnace power supply
GTF Graphite tube furnace
T_1 Tap to plasma torch
T_2 Tap to furnace
P Plasma torch
F_1 Flowmeter valve sample aerosol
F_2 Flowmeter valve carrier gas
N Concentric Nebulizer
B Auxiliary bottled gas argon supply
C Aluminium end cap
A
GTF
T_2
T_1
C
coolant
Ball & Socket joint
Transfer tube
F_1
F_2
aerosol gas
carrier gas
N
Nebulizer # 1 port (Main gas supply)
B

HEALTH EFFECTS OF AIR POLLUTION

P. Corey, F. Silverman, S. Mintz and R. Hosein
Dept. of Medicine, University of Toronto

The health effects of air pollution, particularly at low levels, are still somewhat uncertain. Most studies to date have utilized imprecise assessments of personal exposure to air pollution because of their reliance on pollution data obtained from fixed air pollution stations monitoring outside air. Because individuals can live some distance from such stations and because they spend up to 90% of their time indoors the need for better estimates of exposure has arisen. A personal portable multi-pollutant sampler has been designed at The Gage Research Institute and measures concentrations of nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and suspended particular matter (SPM). Another problem with previous studies has been the use of severe symptoms or signs as measures of health effects while ignoring minor fluctuations in symptoms and pulmonary function. In an attempt to remedy these deficiencies a study was designed to measure the health effects of air pollution.

Thirty asthmatics and fourteen healthy non-asthmatics were involved over the period January 1981 to July 1982. Each subject was visited at his home on up to twenty days both in the morning and afternoon, contributing a total of approximately 500 person-days. On each day pulmonary function was assessed in the morning. Measurements included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and maximum mid-expiratory flow rate ($FEF_{25\%-75\%VC}$).

A personal multi-pollutant sampler was then activated and carried by the individual for the rest of the day up until about 4 P.M. at which time it was shut off and pulmonary function again measured. The two visits on each day permitted not only as estimate of the subject's pulmonary function on a given day but also the change over the day. These pulmonary function tests were administered according to standard procedures. At each session at least 3 maximum expiratory manoeuvres were performed by the subject until 2 spirograms were obtained which were reproducible to within 5% for both flow and volume. The Vitalograph was calibrated monthly for volume using a one litre syringe and time.

Levels of Pollutant Concentration:

The frequency distribution for NO_2 and SO_2 obtained using the sampler carried by the subject (PERSONAL) and by the sampler situated at the Ministry site (GAGE MOE) are shown in Figures 1 to 2. The total number of sampling days is 379.

Within Individual Analysis:

The potential effect of the exposure to NO_2, and SO_2 on pulmonary function was analyzed by means of different strategies listed in Table 1.

A. Relationship Between Daily Change in Pulmonary Function and Daily Pollutant Concentrations.

The first strategy was concerned with an assessment of the relationship between the change in pulmonary function over the day and the corresponding pollutant concentration on that day. This was accomplished by simply calculating for each individual the correlation coefficient and slope of the relationship between daily pollutant concentrations and the corresponding day's change in pulmonary function. For each individual, 4 slopes were calculated, one between each of the two pulmonary function changes and each of the two pollutants measured. For each individual these slopes were based on the same number of days on which complete information was collected on the two pulmonary function changes and the two pollutants. However the number of days on which a slope is calculated will vary among the individuals.

The change in pulmonary function was measured by substracting the afternoon value from the morning value. Therefore this difference will be positive if the pulmonary function decreased over the day. Therefore if the drop in $FEF_{25\%-75\%VC}$ was larger for an individual on those days for which the SO_2 concentration was high, then the slope and correlation coefficient between the daily change in $FEF_{25\%-75\%VC}$ and the corresponding day's SO_2 concentration would be positive. An example is shown for one of the subjects in Figure 3. This subject had complete information on 18 days and the slope between the daily change in $FEF_{25\%-75\%VC}$ and the corresponding day's SO_2 concentration was 0.42. This can be interpreted to mean that an increase in SO_2 concentration of one part per billion will be associated with an additional drop in $FEF_{25\%-75\%VC}$ of 420 ml/sec. The correlation coefficient for these 18 data points is 0.60 ($p<.01$). For each asthmatic and each non-asthmatic a slope was calculated between the daily change in $FEF_{25\%-75\%VC}$ and the daily SO_2 concentrations. If there were no relationship between the daily change in $FEF_{25\%-75\%VC}$ and SO_2 then one would expect half of the slopes to be positive and half to be negative. If, on the other hand, a deleterious relationship exists then one would expect an excess of positive slopes. This hypothesis was tested using the sign test. The test of whether the mean and median of these slopes was different from zero was conducted using the paired t-test and the Wilcoxon signed rank test respectively. These analyses were conducted for both pulmonary functions and both pollutants. The results are shown in Tables 2 and 3.

Only the slope between the daily change in $FEF_{25\%-75\%VC}$ and the daily SO_2 concentration was found to be statistically significant. The median slope is 10.8. That is, for each increase of one part per billion in SO_2 and additional drop of 10.8 ml/sec in $FEF_{25\%-75\%VC}$ could be expected.

B. Relationship Between Daily Mean Pulmonary Function and Daily Pollutant Concentrations.

The statistical analysis in strategy A was concerned with the question of whether the effect of exposure to higher concentrations of the pollutants NO_2, and SO_2 would be manifested in a drop in pulmonary function on that same day. However, only one of the relationships, namely that between SO_2 and $FEF_{25\%-75\%VC}$ was found to be significant. One reason for this finding might be that the effect of higher pollutant concentrations on pulmonary function might be more gradual. Therefore those days on which the pollutant concentration was high may be associated not with a drop in pulmonary function but with a lower pulmonary function generally over the entire day. Under these circumstances we might expect a negative correlation between pulmonary function and pollutant concentration over the days monitored for each subject. Therefore in the same manner as was outlined in strategy A, a correlation coefficient and slope was estimated for each subject. However as the dependent variable, the daily mean pulmonary function was used instead of the daily difference in pulmonary function. The results of these analyses are shown in Tables 4 and 5. As before the sign test was used to test whether there was a significant increase in the proportion of negative slopes. The paired t-test and Wilcoxon signed rank test were used to test whether the mean slope and median slope were significantly negative. No relationships were statistically significant.

Further Within-Individual Analyses:

All of the analyses were repeated with the pollutant concentration being replaced by the logarithm of the pollutant concentration. Because the SO_2 concentration could be as low as zero, a constant value of 1 was added to the SO_2 concentration before using the log transformation. Because only the results obtained between the daily mean $FEF_{25\%-75\%VC}$ and the log NO_2 and log SO_2 concentrations were significant they will be discussed at some length. The results that proved significant were those that used the NO_2 and SO_2 concentrations measured at the ministry site with the Gage sampler (GAGE MOE) and not using the sampler carried by the subject (PERSONAL). Furthermore the following discussion will emphasize the importance of using an analysis that measures the relationship within individuals and not across individuals.

The correlation coefficient between the daily mean $FEF_{25\%-75\%VC}$ and the corresponding log NO_2 concentration was very low, $r=-0.03$. The correlation between $FEF_{25\%-75\%VC}$ and the log SO_2 concentration was also very low, $r=0.01$. Both of these coefficients were based on a total of 379 person-days pooled across 41 subjects.

This analysis was repeated using the individual as the sampling unit and correlating the mean daily $FEF_{25\%-75\%VC}$ of an individual with his mean log NO_2 and mean log SO_2 concentration averaged over the same number of days. This analysis was based on the same 41 subjects and again the correlation coefficients were very low, being -0.01 for the relationship between an individual's mean $FEF_{25\%-75\%VC}$ and mean log NO_2 and r = C.17 for the relationship between the individual mean $FEF_{25\%-75\%VC}$ and mean log SO_2. However as before a slope was calculated for each individual for the relationship between the daily mean $FEF_{25\%-75\%VC}$ and the corresponding day's log NO_2 and log SO_2 concentration. A scattergram of the relationship between $FEF_{25\%-75\%VC}$ and log NO_2 for one particular subject is shown in Figure 4. As seen for this subject, there is a strong negative relationship between the daily mean $FEF_{25\%-75\%VC}$ and the daily mean log NO_2 concentration. The slope was -0.64 ml/sec/log ppb and the correlation coefficient was -0.77 ($p<0.01$). There were N = 39 individuals that had at least two complete days of information and for whom slopes could be calculated. If there were no relationship between daily mean $FEF_{25\%-75\%VC}$ and daily log NO_2 then one would expect half of the slopes to be negative and half positive. However as shown in Figures 5 and 6, the mean slope for $FEF_{25\%-75\%VC}$ and log NO_2 was significantly negative, -86 ml/sec/log ppb ($p<.05$). Similarly the mean slope between $FEF_{25\%-75\%VC}$ and log SO_2 was also significantly negative, -71 ml/sec/log ppb ($p<.05$). Although these two results were not dramatic, the p value being only less than 0.05, they do illustrate that quite divergent results can be obtained by analyzing results within individuals rather than either pooling results across person-days or analyzing across individuals the relationship between individual mean $FEF_{25\%-75\%VC}$ and the corresponding mean pollutant concentration.

Between Individual Analysis:

As indicated in Table 1 two major types of statistical analyses were used to assess the relationships between the exposure variables and the response variables. The first type reported above analyzed changes within individuals. The second type analyzed relationships across individuals. This analysis is designed to answer the question - do individuals who are exposed to high pollutant concentrations exhibit larger response.

One reason that this type of analysis might improve the chance of detecting a relationship is that the range of pollutant concentrations to which different individuals are exposed might be larger than that expected for a single individual. If this were so then the reliability of slopes for the between individual analysis could be expected to be higher than that for the within individual analysis. To test this possibility an analysis of variance of the two pollutant variables was performed to determine the relative sizes of the variance between individuals and the variance within individuals and found the variance within individuals to be much larger than that between individuals. Therefore if the between individual analysis is to prove more informative it will not be due to the increased range of pollutant concentrations faced by different individuals. In fact the larger variance of the pollutant concentrations within individuals probably reflects the measurement error of the monitoring network which can be decreased by using mean values of individuals rather than the single measurements. Therefore using mean values of individuals as the basic sampling unit for analysis might improve the analysis because of the increased reliability of such mean values relative to the single observations that comprise it.

C. Relationship Between An Individual's Mean Change in Pulmonary Function and His Overall Mean Pollutant Concentrations.

For each individual the mean concentration of NO_2 and SO_2 was calculated over the number of days in the study that the subject had complete data on both pollutant concentration and pulmonary function. Over the same sequence of days, the mean daily change in pulmonary function was calculated, that is, the mean of the morning value minus the mean of the afternoon value. As in analysis A if the pollutant has a deleterious effect on pulmonary function then we would expect individuals exposed to high pollutant concentrations to have a larger positive difference in pulmonary function over the day. Only for NO_2 is there any indication of an effect (6). The correlation coefficient between the mean NO_2 concentration and the mean change in FEV_1 is 0.26 (p=.08) and with the mean change in $FEF_{25\%-75\%VC}$ is 0.33 (p=.03). However in both cases the statistical significance is substantially reduced when percentage change in FEV_1 and $FEF_{25\%-75\%VC}$ are used instead of linear change, the correlation coefficients being 0.17 (p=.27) for FEV_1 and 0.22 (p=.16) for $FEF_{25\%-75\%VC}$.

Repeating this analysis for the asthmatics and non-asthmatics separately revealed interesting differences (7). The relationship between mean NO_2 concentration and mean change in FEV_1 was much stronger in the group of non-asthmatics, the correlation coefficient being 0.61 (p=.02) whereas it was only 0.19 (p=.31) for the asthmatics.

D. Relationship Between An Individual's Overall Mean Pulmonary Function and His Overall Mean Pollutant Concentrations.

As in analysis D, the mean concentration of NO_2 and SO_2 was calculated for each individual over those days on which complete pollutant and pulmonary function data was collected. For each individual over the same sequence of days the mean pulmonary function measurement was calculated. If the pollutant has a deleterious effect on pulmonary function then one would expect an inverse relationship between mean pollutant concentration and mean pulmonary function. The results are reported in Tables 8 and 9.

The positive correlation between NO_2 and FEV_1 is similar for both asthmatics (r=.39) and non-asthmatics (r=.35) although only significant for asthmatics.

The positive correlation of 0.45 between NO_2 and $FEF_{25\%-75\%VC}$ is only significant for the asthmatics. Only for asthmatics is the correlation coefficient between SO_2 and FEV_1 (r=0.40) significant. These results illustrate the serious problems of interpretation that can be encountered in the analysis of cross-sectional data which is what analysis E actually is. We are attempting to estimate relationships across individuals by taking single measures for each variable in the relationship. Again we do not interpret these results to mean that exposure to high concentrations of NO_2 will improve a patient's FEV_1 but rather that the less healthy asthmatic with the lower FEV_1 will possibly modify his environment so as to reduce the NO_2 concentration to which he is exposed. The same interpretation could be applied to the other significant and positive relationships found in Table 8 and 9.

SUMMARY

1. The choice of scale used for the pollutant concentration can affect the relationship between pollutant and lung function.
2. Pollutant concentration may be related to the average daily pulmonary function or to the change in pulmonary function over the day.
3. The choice of sampling unit can be very important. Relationships between pollutant levels and lung function within individuals can be masked by analyses across individuals or by analyses that pool the data from all of the individuals.

TABLE 1. STRATEGIES OF ANALYSIS

1. WITHIN INDIVIDUAL ANALYSIS
    - (A) Relationship between daily change in pulmonary function and daily pollutant concentration
    - (B) Relationship between daily mean level of pulmonary function and daily pollutant concentration.

2. BETWEEN INDIVIDUAL ANALYSIS
    - (C) Relationship between an individual's mean change in pulmonary function and his overall mean pollutant concentration
    - (D) Relationship between an individual's overall mean pulmonary function and his overall mean pollutant concentration.

TABLE 2. RELATIONSHIP BETWEEN DAILY CHANGE IN FEV_1, $FEF_{25\%-75\%VC}$ AND DAILY NO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of positive slopes	0.51	0.51
Mean slope*	1.30	0.03
Median slope	0.05	1.63

*Slopes are reported as changes in pulmonary function (ml or ml/sec) per part per billion of pollutant.

TABLE 3. RELATIONSHIP BETWEEN DAILY CHANGE IN FEV_1, $FEF_{25\%-75\%VC}$ AND DAILY SO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec
Number of individuals	43	43
Proportion of positive slopes*	0.51	0.63
Mean slope	-4.00	31.40
Median slope	0.14	10.8**

*Slopes are reported as changes in pulmonary function (ml or ml/sec) per part per billion of pollutant.

**$p<0.05$

TABLE 4. RELATIONSHIP BETWEEN DAILY MEAN PULMONARY FUNCTION AND DAILY NO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of negative slopes	0.42	0.52
Mean slope*	2.20	3.60
Median slope	0.80	-0.12

*Slopes are reported as change in daily mean pulmonary function (ml or ml/sec) per part per billion of pollutant.

TABLE 5. RELATIONSHIP BETWEEN DAILY MEAN PULMONARY FUNCTION AND DAILY SO_2 CONCENTRATION

	Pulmonary Function	
	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (ml/sec)
Number of individuals	43	43
Proportion of negative slopes	0.47	0.47
Mean slope*	6.00	-7.80
Median slope	1.63	0.90

*Slopes are reported as changes in daily mean pulmonary function (ml or ml/sec) per part per billion of pollutant.

TABLE 6. CORRELATION COEFFICIENT BETWEEN MEAN POLLUTANT EXPOSURE AND MEAN CHANGE IN PULMONARY FUNCTION (N*=44)

	Δ FEV$_1$ (1)**%	Δ FEF$_{25\%-75\%VC}$ (1/sec)
NO$_2$	0.26 p = .08	0.33 p = .03
SO$_2$	0.13 p = .40	-0.01 p = .96

*N = number of persons
**Δ = linear change

TABLE 7. CORRELATION COEFFICIENTS BETWEEN MEAN POLLUTANT EXPOSURE AND MEAN CHANGE IN PULMONARY FUNCTION

	Δ FEV$_1$ (1)**%	Δ FEF$_{25\%-75\%VC}$ (1/sec)
	Non-Asthmatics (N* = 14)	
NO$_2$	0.61 p = .02	0.37 p = .19
SO$_2$	0.78 p = .001	0.29 p = .32
	Asthmatics (N* = 30)	
NO$_2$	0.19 p = .31	0.33 p = .07
SO$_2$	-0.06 p = .77	-0.19 p = .32

*N = number of persons
**Δ = linear change

TABLE 8. CORRELATION COEFFICIENTS BETWEEN MEAN POLLUTANT EXPOSURE AND MEAN LEVEL OF PULMONARY FUNCTION (N^* = 44)

	FEV_1 (1)	$FEF_{25\%-75\%VC}$ (1/sec)
NO_2	0.43	0.32
	p = .004	p = .03
SO_2	0.38	0.24
	p = .01	p = .11

*N = number of persons

TABLE 9. CORRELATION COEFFICIENTS BETWEEN MEAN POLLUTANT LEVEL AND MEAN LEVEL OF PULMONARY FUNCTION

	Non-Asthmatics (N^*=14)		Asthmatics (N^*=30)	
	FEV_1(1)	$FEF_{25\%-75\%VC}$ (1/sec)	FEV_1(1)	$FEF_{25\%-75\%VC}$ (1/sec)
NO_2	0.35	0.03	0.39	0.45
	p = .22	p = .92	p = .03	p = .01
SO_2	0.27	0.04	0.40	0.30
	p = .34	p = .89	p = .03	p = .11

*N = number of persons

FIGURE 1. FREQUENCY DISTRIBUTIONS

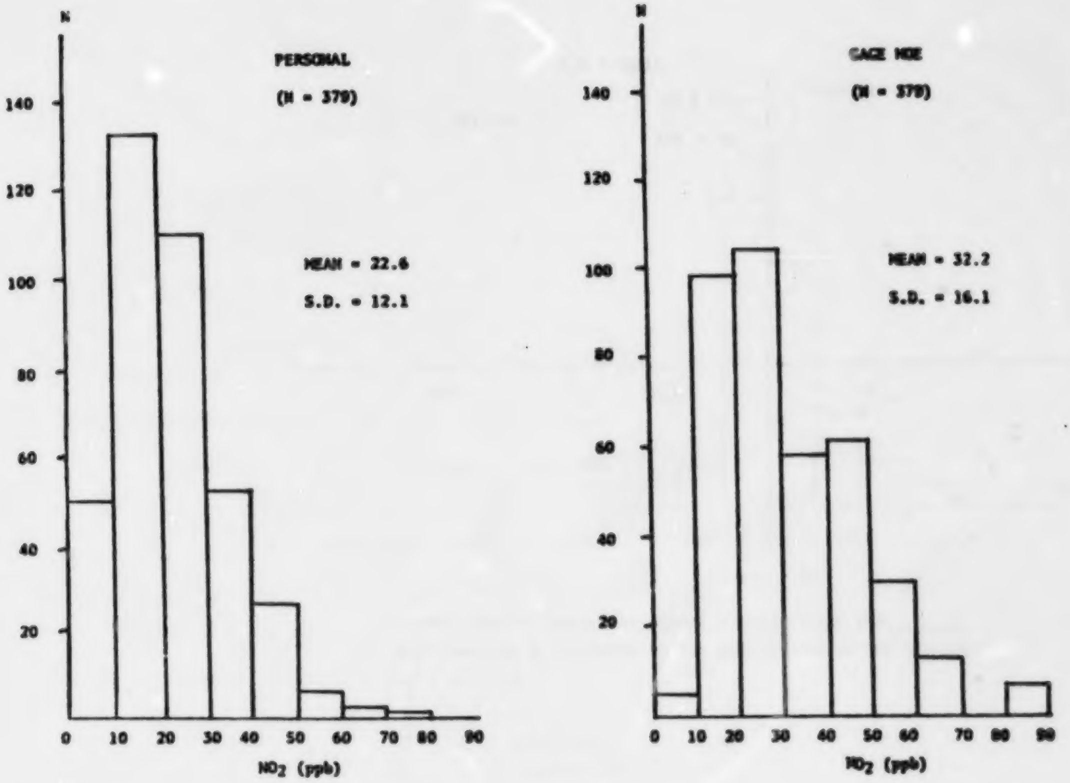


FIGURE 2. FREQUENCY DISTRIBUTIONS

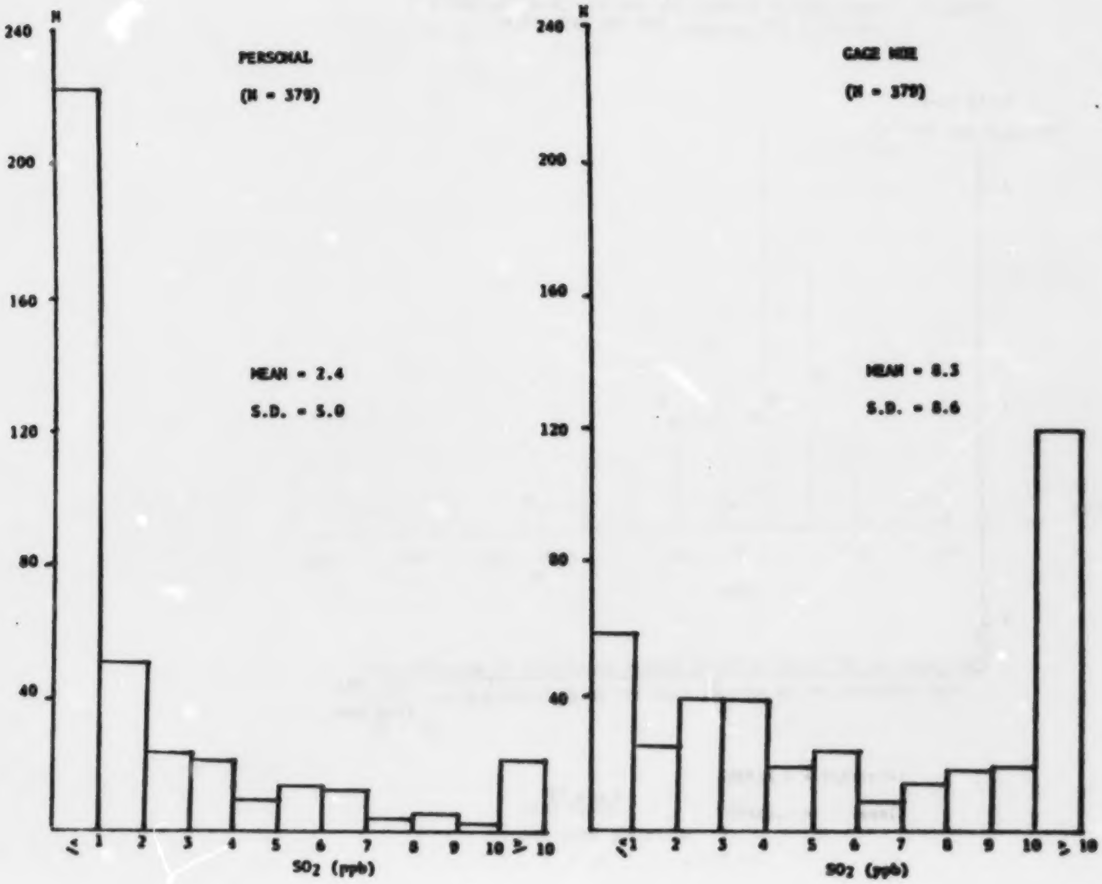


FIGURE 3. RELATIONSHIP BETWEEN SO_2 CONCENTRATION AND DAILY CHANGE IN $FEF_{25\%-75\%VC}$ FOR ONE INDIVIDUAL

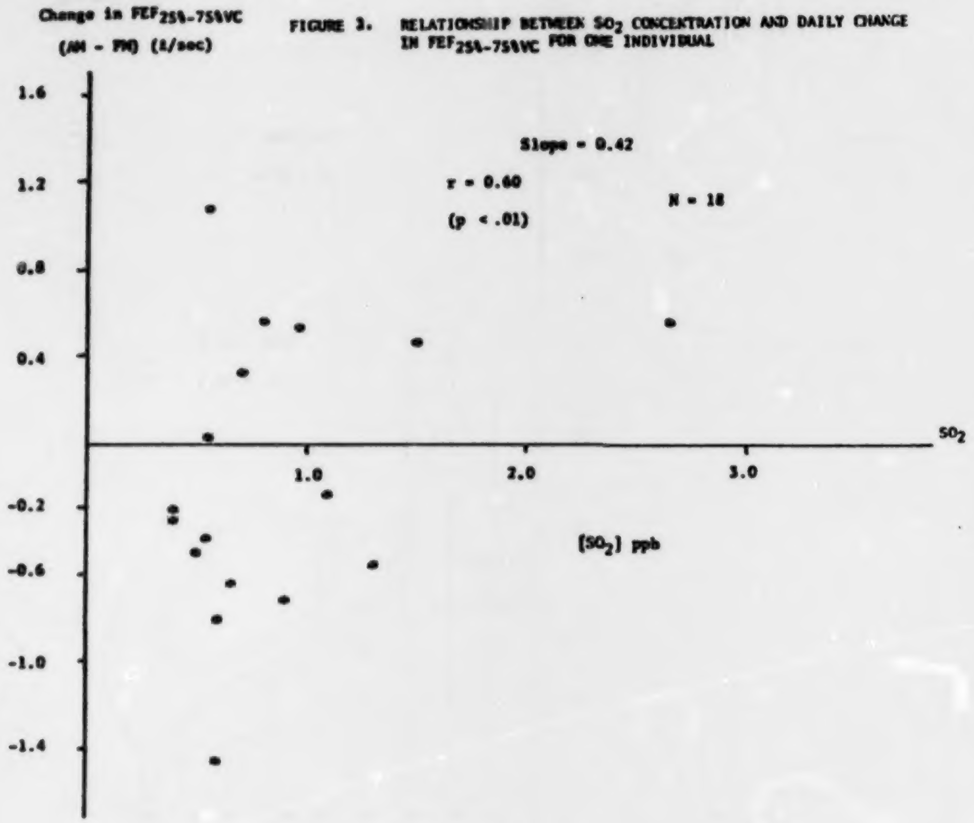
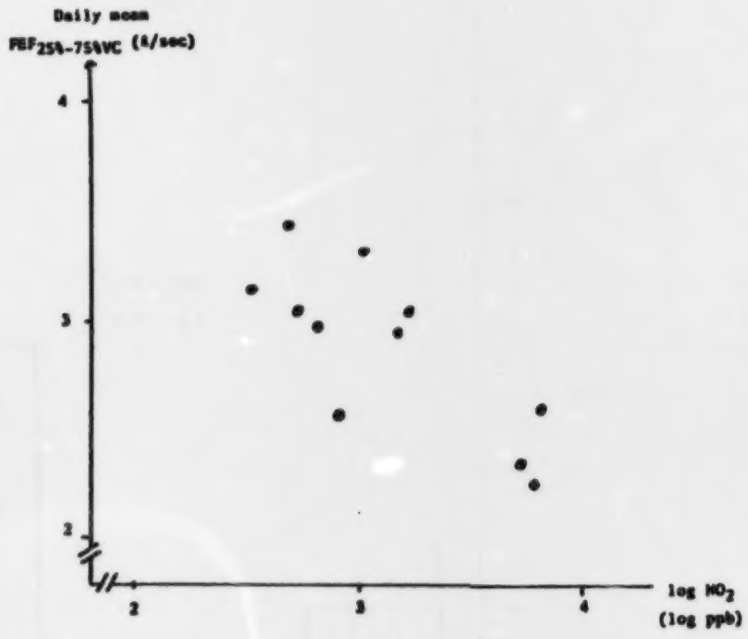


FIGURE 4. RELATIONSHIP BETWEEN THE LOG NO_2 CONCENTRATION AND THE DAILY $FEF_{25\%-75\%VC}$ FOR ONE INDIVIDUAL

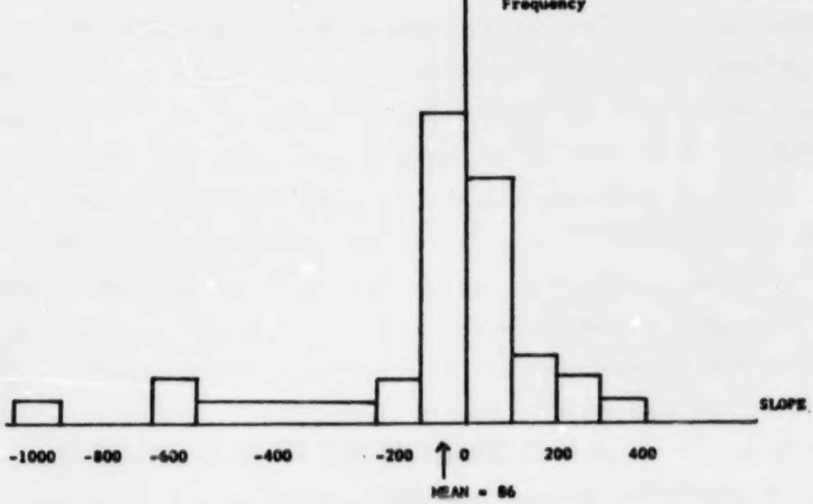


Intercept = 4.858867

Slope = -.63832

R = -.77

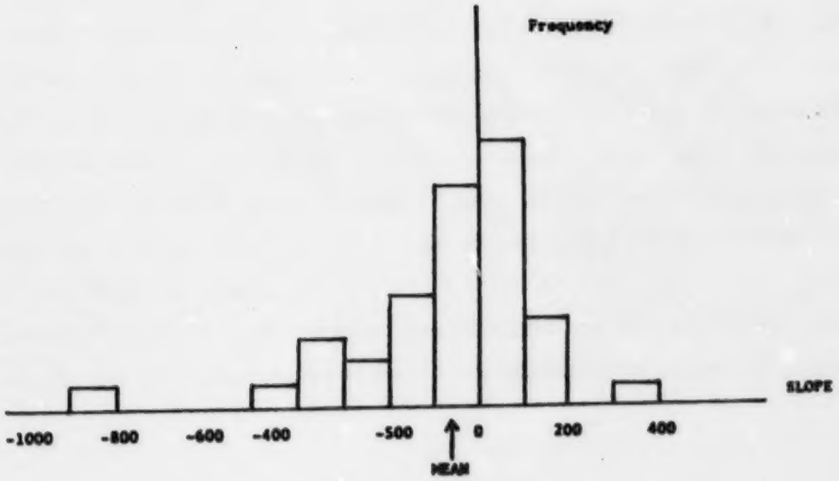
FIGURE 5.



DISTRIBUTION OF INDIVIDUAL SLOPES RELATING DAILY $FEF_{25\%-75\%VC}$ AND DAILY LOG NO_2 MEASURED BY THE GAGE MONITOR AT THE MINISTRY SITE (N = 39).

MEAN SLOPE -86
STANDARD DEVIATION 0.26
t = -2.1 p = 0.05

FIGURE 6.



DISTRIBUTION OF INDIVIDUAL SLOPES RELATING DAILY $FEF_{25\%-75\%VC}$ AND DAILY LOG SO_2 MEASURED BY THE GAGE MONITOR AT THE MINISTRY SITE (N = 39).

MEAN SLOPE -71
STANDARD DEVIATION 0.20
t = -2.16 p = 0.04

A MASS SPECTROMETRIC STUDY OF SELECTED AIR POLLUTANTS

Dr. R.E.March and R.J.Hughes

Dept. of Chemistry,
Trent University,
Peterborough,Ontario

ABSTRACT:

Among the growing list of environmental pollutants present in the atmosphere is a class of multiple fused-ring aromatics known as Polycyclic Aromatic Hydrocarbons (PAH). Only a small number of these PAHs and related heterocycles such as anthracene,pyrene and carbazole are produced in pure form for industrial purposes, however many are produced whenever organic material is heated above 700 ° C, such as in pyrolysis or incomplete combustion. Furthermore, if the starting materials contain heteroatoms such as nitrogen,oxygen and sulphur the the products will contain a variety of related heteroaromatic compounds.

In general, PAHs are found everywhere in the environment and concern over their presence stems from the carcinogenicity of some members of this group such as benzo[a]pyrene. More recently concern has grown due to a degree of excess carcinogenicity from both urban air and exhaust particulates from combustion engines, which could not be accounted for solely on the basis of their measured content of benzo[a]pyrene and other related PAH. It has now been shown that PAHs can combine with other priority pollutants such as nitrogen oxides(NO_x), sulphur oxides(SO_x), ozone(O_3) and peroxy acetylnitrate(PAN). Furthermore this process may be facilitated by the adsorption of PAHs onto the surface of small particulate organic matter(POM) in the respirable(1 micron) diameter range. Thus the problems in urban atmospheres can be compounded by the production, from inactive PAH, of substituted PAHs which are active in the Ames microsomic mutagenicity test.

It has been found that the reaction of benzo[a]pyrene with NO_2 at 300 ° C gives rise to a variety of products including the mono-,di- and trinitro-derivatives which are presumably mutagenic in nature, as well as quinone derivatives some of which may be active and some of which are not. Prolonged heating of benzo[a]pyrene(12 hr.) with a molar excess of NO_2 in the ratio 5:1 or greater appears to result in the production of a lactone-quinone of molecular weight 300 amu. whose activity is unknown.

The storage of positively-charged benzo[a]pyrene and chrysene ions in the presence of NO and NO_2 has shown them to be unreactive under the conditions employed.

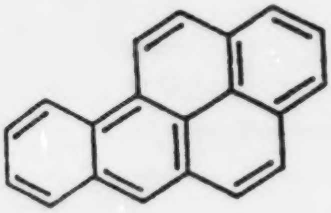
Introduction

A great deal of attention has been given over the past few decades to the major pollutants present in urban atmospheres. These include sulphur oxides,carbon monoxide,nitrogen oxides,photochemical oxidants and particulate matter. Recently however, interest has developed in better understanding the presence, transport and chemical properties of a group of so-called "non-criteria pollutants" which, although present in urban atmospheres at lower concentrations than the "criteria pollutants", are known to cause disproportionately severe health effects in experimental animals and possibly in man[1]. One example of a class of such pollutants is the Polycyclic Aromatic Hydrocarbons(PAH) which are produced whenever there is pyrolysis or incomplete combustion of organic matter. PAHs consist of three or more fused benzene rings in linear, angular or cluster arrangements as shown in Figure 1, and contain only carbon and hydrogen. Although only 8 are shown here there are as many as 200 different PAHs in the air, soil and water[2].

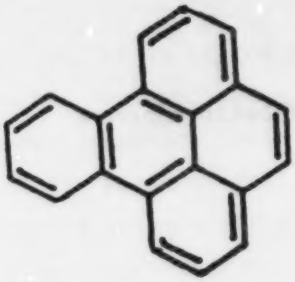
PAH formation proceeds by free radical mechanisms; radical species can combine rapidly at high temperatures or under pyrolytic conditions; reactive transients are stabilized by ring closure, condensation, dehydrogenation, Diels-Alder reactions, ring expansions and other pathways to yield complex polycyclic organic matter[3] such as benzo[a]pyrene depicted in Figure 2.

In Figure 3 is shown the reaction of benzo[a]pyrene in biological systems. The starting material, once into the body, is oxidized to a form known as the "ultimate carcinogen", an epoxy-diol which can then attach itself to a molecule of DNA with the resulting biological effect of cancer initiation.

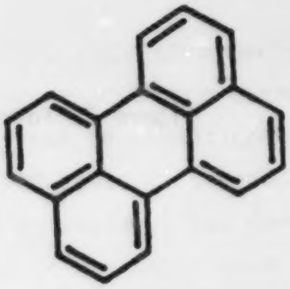
There is an appreciable amount of information in the literature about the biological reactions of many PAHs as well as the analytical methods employed for their detection. Unfortunately there is not only a lack of reliable information on atmospheric transformations of these chemicals but some actual misinformation: in two papers, from 1971[4] and 1976[5] it is reported that PAHs as air pollutants are "chemically inert and are thus removed from the air only by rain or the slow sedimentation of the particulate." Although the evidence is limited it is clear that many polycyclics, such as benzo[a]pyrene, are highly reactive compounds.These undergo a variety of atmospheric reactions both thermal and photochemical, with a number of co-pollutants. Furthermore, there is some evidence thatthese reactions may be facilitated by adsorption onto particulate matter in the respirable(1 micron) diameter range. In a 1980



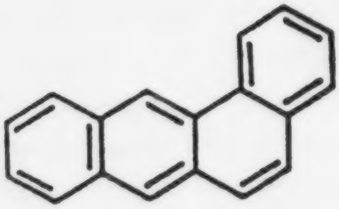
Benzo[a]pyrene



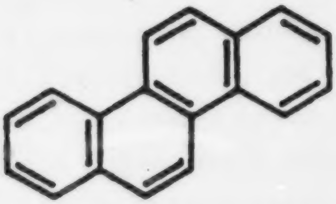
Benzo[e]pyrene



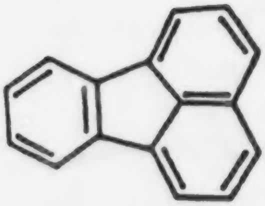
Perylene



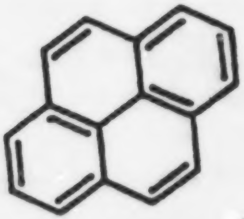
Benz[a]anthracene



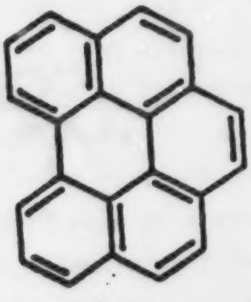
Chrysene



Fluoranthene



Pyrene



Benzo[ghi]perylene

Figure 1. Polycyclic Aromatic Hydrocarbons Present in Combustion-Related Particulate Organic Matter.

Figure 2. Formation of benzo[a]pyrene

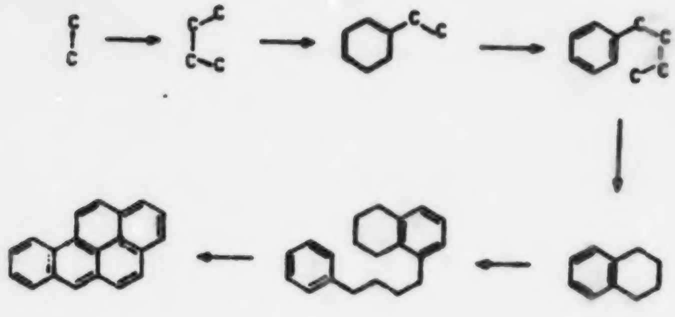
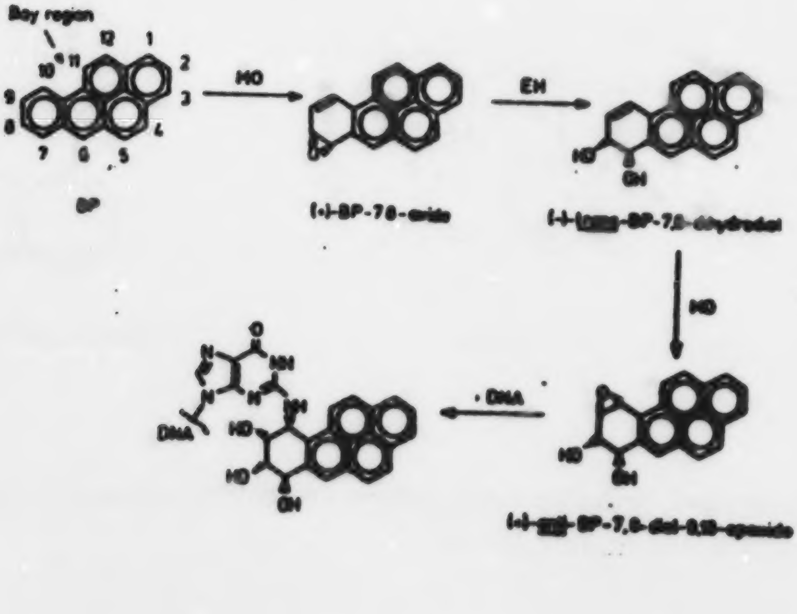


Figure 3. Biological oxidation of benzo[a]pyrene
MO is MonoOxygenase EH is EpoxyHydrolase


Bay region
12
1
2
3
4
5
6
7
8
9
10
11
MO
EH
BP
(+)-BP-7,8-oxide
HO
OH
(−)-trans-BP-7,8-dihydrodiol
MO
+ DNA
(+)-anti-BP-7,8-diol-9,10-epoxide
O
N
NH
N
N
NH
DNA
HO
HO
OH

paper by Dr. J.N.Pitts et al, it is stated that" First, although organic extracts of polycyclic organic matter from ambient air and the exhaust from spark ignition engines are known to be carcinogenic, their activity can be significantly greater than would be expected on the basis of their known PAH content. Second, application of the Ames Salmonella mutagen assay to such extracts... has demonstrated a significant level of direct mutagenicity ... which is not ascribable to the promutagenic PAH."

Experimental Work and Results

Preliminary studies have been conducted of the two PAH benzo[a]pyrene and chrysene. The conventional electron impact mass spectra of these compounds obtained on an AEI MS12 magnetic sector mass spectrometer are quite similar. Both show a large peak corresponding to the molecular ion at 252 amu for benzopyrene and 228 amu for chrysene. These are accompanied by peaks at M-26 and M-52 indicating loss of one and two ethylene units, respectively, and the associated metastable peaks. The stability of these ions is evidenced by the fact that there also appear on the spectra peaks associated with doubly,triply and even quadruply-charged ions as well as doubly-charged metastable peaks.

Neither substance shows any change upon heating to 400°C in vacuo. In the presence of oxygen, both compounds react little at 100°C but are severely degraded upon heating to 400° for 12 hours.

Experiments were performed involving chrysene and oxygen below the ratio of 21:1 which would theoretically give only CO_2 and H_2O. All samples were heated to 400°, held for 4 hours and allowed to cool. All samples gave similar spectra regardless of the amount of oxygen present indicating that although reaction occurs in oxygen-rich conditions after 12 hours, there is little reaction in oxygen-lean conditions after 4 hours.

In the vapour condensed from the reaction tubes there were observed peaks corresponding to water, carbon dioxide and phenol.There were no traces of oxygen atoms or molecular oxygen being added to the parent molecule upon heating but rather a change in the fragmentation pattern which could indicate a skeletal rearrangement; peaks at m/z 215,216 and 217 which had been present in the mass spectrum of the starting material disappeared and a new peak at 218 appeared. There was also a decrease of m/z 242 and 266(impurities) and an increase in 246 and 280 possibly due to formation of higher-molecular-weight PAH.

In the case of benzo[a]pyrene when irradiated with ultraviolet light in the presence of oxygen, two hydrogen atoms are replaced by oxygen resulting in the formation of benzo[a]pyrene quinone isomers.

Experiments were also performed in sealed tubes containing 2.5mg PAH and nitrogen dioxide in 1:1 and 1:10 ratios which were heated to 300°C and held overnight.The resulting mass spectra showed a variety of reaction products from both chrysene and benzo[a]pyrene and are reproduced in Figures 4 and 5.

In a 1:1 ratio with nitrogen dioxide chrysene produces a number of mass spectral peaks which include the mononitro derivative at m/z 273 and two related peaks at 243 and 215 which are either simple electron impact fragments or the stable products chrysene ketone and the ketone minus carbon monoxide. The base peak m/z 230 is presumably a rearrangement product of chrysene. The fragmentation pattern in the spectrum at m/z 224 to 227 probably indicates the presence of a number of mononitro isomers.

In a 1:10 ratio with nitrogen dioxide the sample was severely degraded and the mass spectrum, shown in Figure 4(c), contained large peaks at m/z 50,76, 104,medium peaks at m/z 122,148 and 168, and all other peaks being less than 1% of the base peak.

The electron impact spectrum of benzo[a]pyrene is reproduced in Figure 5(a).The reaction with a 1:1 ratio with NO_2 gave rise to the spectrum 5(b). Reaction in a 1:10 ratio produced the spectrum 5(c) which, surprisingly, consisted of only 4 large peaks in excess of m/z 170 and a few minor fragment peaks of low intensity. A series of reactions were then carried out in which the ratio of NO_2 was reduced successively in order to investigate more closely the reactions taking place. The analyses of these products were used to create the profile of reaction products in Figure 6.

On the ordinate of Figure 6. is plotted the height of the mass spectral peak of interest divided by the sum of heights of the other peaks in the spectrum above m/z 170, thus giving the intensity of each peak as a percentage of the total ionization. The lower mass ions were common to all the spectra and were omitted to simplify the calculations. These percentages are plotted against the molar ratio of nitrogen to benzo[a]pyrene in order to portray the products as a function of the amount of NO_2 present.

The above results were obtained after heating for 12 hours;benzo[a]pyrene however begins to react quickly with nitrogen dioxideand visibly darkens at room temperature in a few minutes. A[illegible]0°C this PAH is a colorless vapour but in the presence of NO_2 rapidly becomes a black liquid. Mass spectra of products formed just an hour after the start of the reaction(at 300°) indicate the same peaks present as in the 12-hour reaction but with the additional presence of the tri-

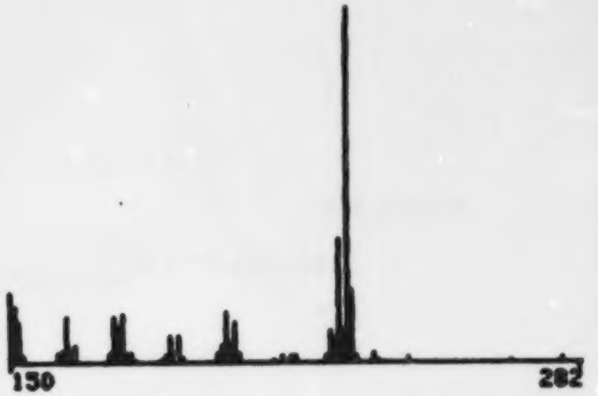


Figure 4(a).

CHRYSENE

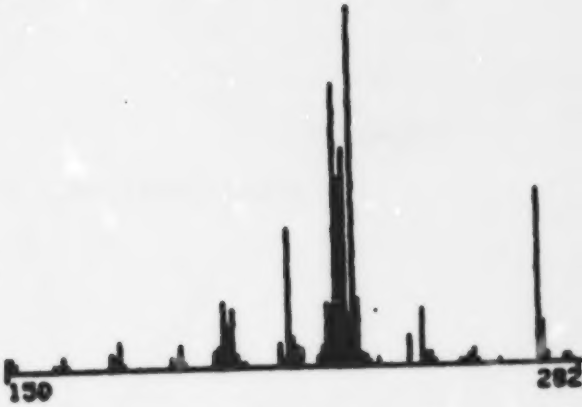


Figure 4(b).

CHRYSENE:NO_2
1:1

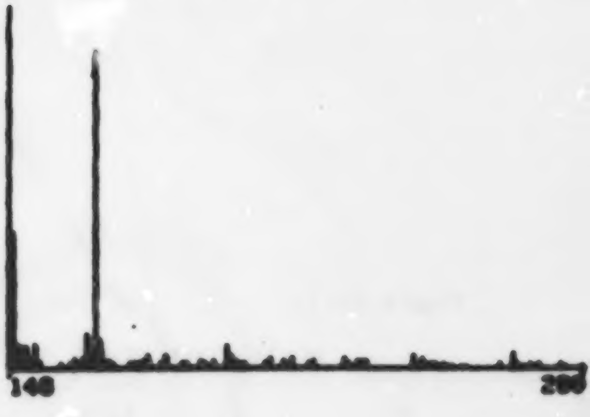


Figure 4(c).

CHRYSENE:NO_2
1:10

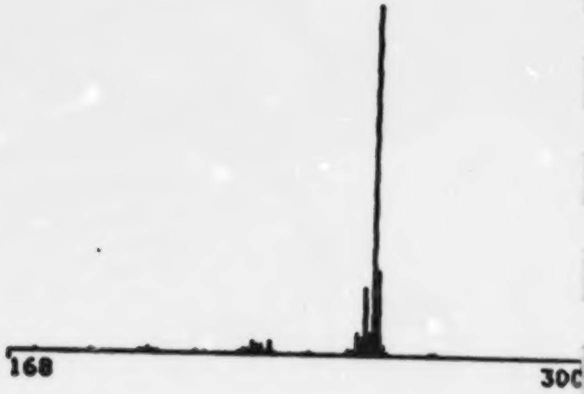


Figure 5(a).

BENZO[a]PYRENE

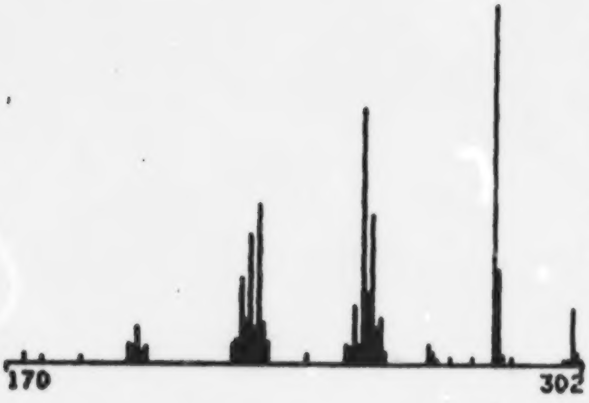


Figure 5(b).

BENZO[a]PYRENE:NO_2

1:1

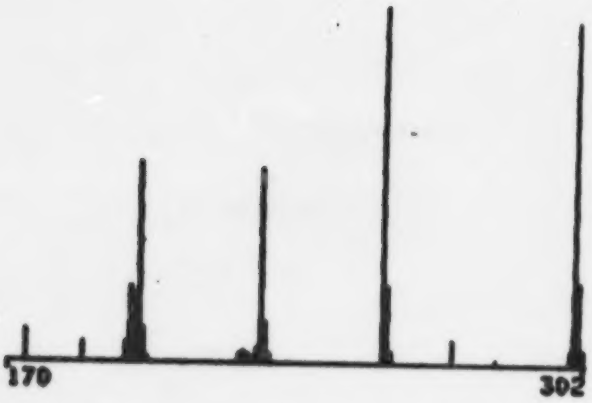


Figure 5(c).

BENZO[a]PYRENE:NO_2

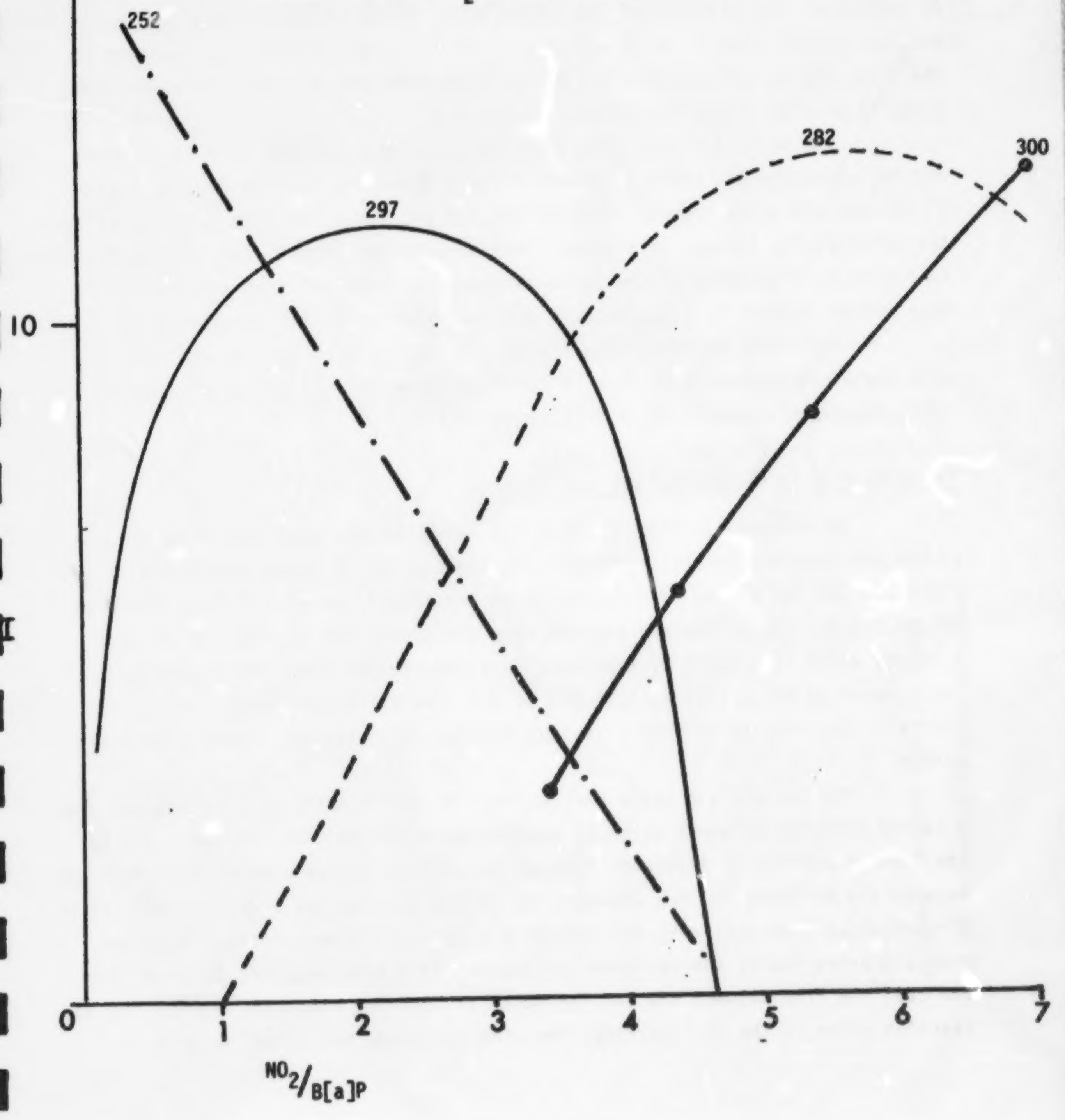
1:10

Figure 6.

Individual Ion Intensity(as % of total)

vs.

Molar Ratio of NO_2 to Benzo[a]pyrene



nitro and dinitro derivatives in addition to the mononitro product. The proposed reaction scheme which accounts for the major ion peaks in the spectra is reproduced in Figure 7. The ion peaks at m/z values of 252,297,342 and 282 represent the stable molecules benzo[a]pyrene,mononitro- and dinitro- benzo[a]pyrene and benzo[a]pyrene quinone dimers, respectively. At ambient temperature the major amount of nitration occurs at the 6- position[7] with minor amounts at the 1- and 3- positions. The position of the second nitro group in the dinitro derivative has not been established so it is not shown, in fig.7, attached at any position on the ring. The structures with m/z 267,312,239,284a and 254a may represent stable products or simply electron impact fragments.

The major ion resulting from the overnight reaction of benzo[a]pyrene and NO_2 where the NO_2 ratio is greater than 5 times that of the PAH has a mass of 300 amu and gives rise to peaks at 256,228 and 200 along with metastables for the accompanying losses. This product may be produced through the pathway depicted in Figure 8. Production of the lactone-quinone of mass 300 from the epoxide of mass 300 is similar to a thermolysis process reported in the literature[9].

Not shown in any of the figures but observed in some of the spectra were peaks corresponding to ions of masses between 480 and 520 which were possibly the dimer products of benzo[a]pyrene and NO_2.

Ion Chemistry of Benzo[a]pyrene and Chrysene

An attempt was made to study the ion/molecule reactions of benzo[a]-pyrene and chrysene in the quadrupole ion storage device shown schematically in Figure 9. The ion store, or QUISTOR, is an ion trap in which the inner surfaces of the top end cap,bottom end cap and ring electrodes are machined to produce a cavity which is bounded by hyperboloids of revolution about the z-axis;aplication of an RF voltage at 1.25 MHz to the ring electrode causes ions in the centre of the trap to undergo Lissajous-type trajectories and remain within the cavity.

The top end cap has a central hole to permit entrance of electrons from a heated tungsten filament which is mounted above the QUISTOR. The emission of the 70eV electrons is regulated in a pulsed fashion by means of a gate electrode between the filament and top end cap; the bottom end cap has a central hole in it through which ions exit into the source of a quadrupole mass filter. Positive ions are extracted by the periodic application of a 50μs negative pulse to the end cap; the time between the initial pulse of ionizing electrons and the ion extraction pulse is the storage time. The ring electrode has a 3mm. hole in it

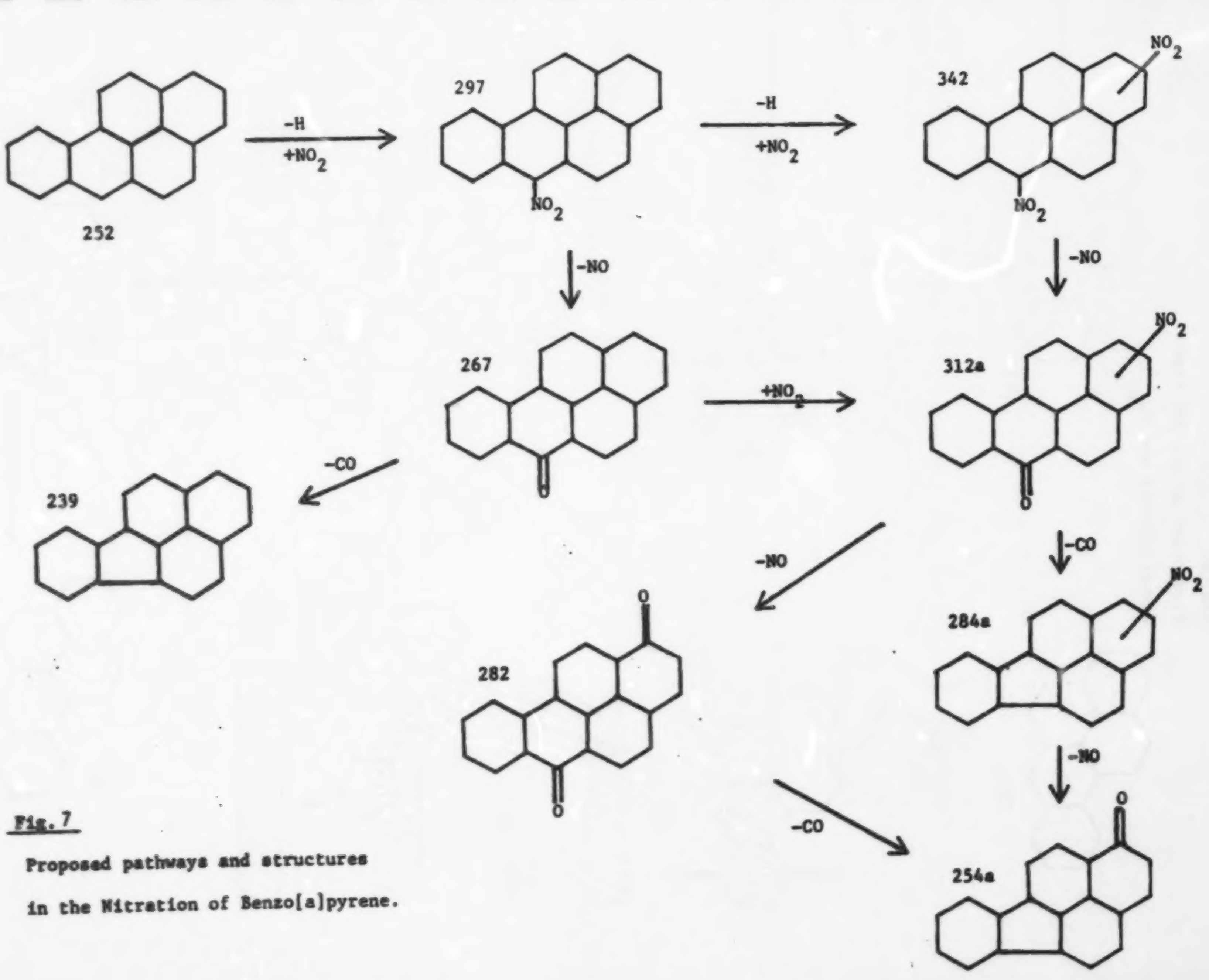

252
-H
+NO$_2$
297
NO$_2$
-H
+NO$_2$
342
NO$_2$
NO$_2$
-NO
-NO
267
+NO$_2$
312a
NO$_2$
O
O
-CO
239
-NO
-CO
282
O
O
284a
NO$_2$
-NO
-CO
254a
O

Fig. 7

Proposed pathways and structures

in the Nitration of Benzo[a]pyrene.

Fig. 8

Production of m/z 300 from Benzo[a]pyrene and NO$_2$

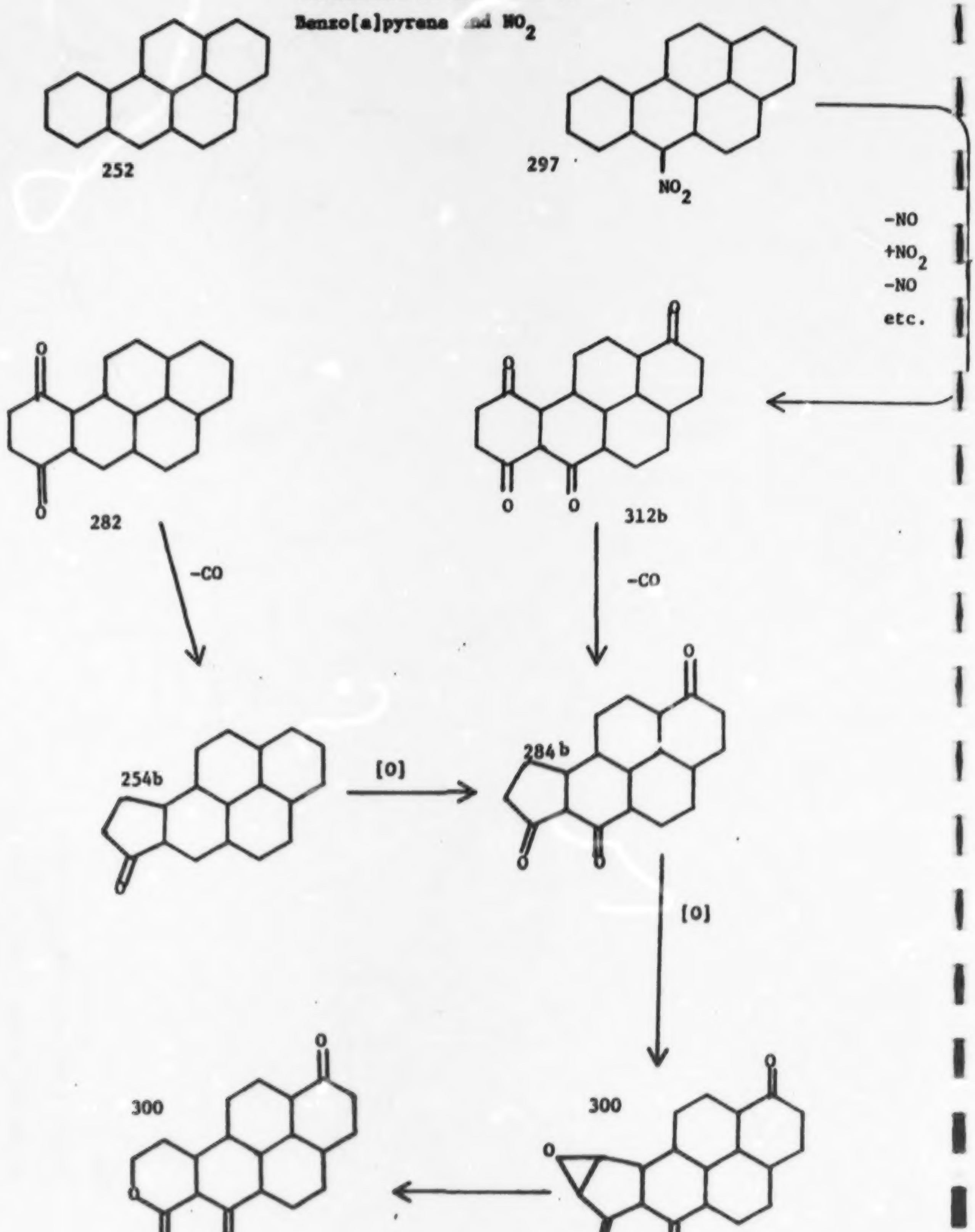
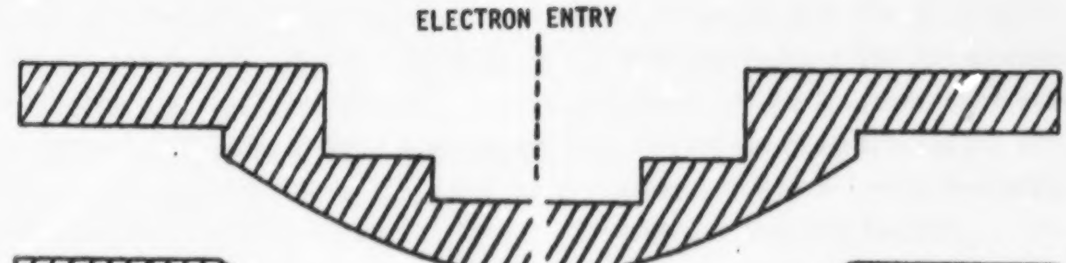

252
297
NO2
-NO
+NO2
-NO
etc.
282
312b
-CO
-CO
254b
[O]
284b
[O]
300
300

Figure 9. Quadrupole Ion Store QUISTOR


ELECTRON ENTRY
Z - AXIS
SAMPLE PROBE ENTRY
ION EXIT

which is aligned with an external viewport in the vacuum tank. Two O-rings mounted at either end of this port form a seal against a 4.76mm. brass rod which can slide in and out of the tank while maintaining a pressure of about 0.02 mPa; affixed to the end of the brass rod is a Pyrex capillary tube fused just above one end to form a shallow depression. When a solid is placed in the depression it can be slid into the QUISTOR through the ring electrode where the ambient temperature vapourizes enough sample to obtain a mass spectrum employing either the electron impact source of the quadrupole mass filter or the QUISTOR in its storage mode.

Storage of ions produced by electron impact of the pure compounds benzo[a]pyrene and chrysene for periods up to 120 ms at pressures up to .5 mPa show very little reaction takes place in these systems. The parent ion accounts for more than 90% of the total ion signal. The smaller peaks are extracted from the background by repeated acquisition using a CYBORG ISAAC 91A digital interface and signal averaging employing an APPLE //e microcomputer.

Each PAH was subsequently stored in the QUISTOR at about 0.5 mPa and nitrogen dioxide or nitric oxide was admitted to a pressure of 10 mPa. The only change in the spectra observed was an increase in a peak at m/z 254 when NO was admitted with chrysene. This peak represented only about 0.25% of the total ionization and was only observed after signal averaging 100 sweeps of the quadrupole at high sensitivity. None of the results observed in these systems indicated a significant amount of reactivity of PAH ions under these conditions.

The lack of reactivity among these PAH ions is understandable on the basis of their aromatic character. The number of rings present in the system delocalize the electronic charge on the ion giving rise to enhanced stability. This stabilization effect is evident in the conventional electron impact spectra which displays quadruply-charged ions and multiply-charged metastables.

It is safe to say that some Polycyclic Aromatic Hydrocarbons, specifically benzo[a]pyrene and chrysene, are not inert in the presence of co-pollutants such as nitrogen dioxide, or oxygen and ultraviolet light in concert. Although their ions are not reactive under the conditions employed herein, it appears that these two, and probably many more polycyclics are highly reactive in the vapour phase and produce a large number of gaseous products, some of which will undoubtably prove to be harmful.

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DEVELOPMENT OF A METHOD FOR MEASURING HYDROGEN PEROXIDE USING A TUNABLE DIODE LASER ABSORPTION SPECTROMETER

D.R. Hastie and H.I. Schiff

Department of Chemistry, York University

ABSTRACT

Hydrogen peroxide is believed to be the most important oxidant in transferring SO_2 to H_2SO_4. It is also closely coupled to the highly reactive HO and HO_2 radicals which are central to all chemical processes in the atmosphere. Measurements of the gaseous concentration of this species are therefore important in furthering our understanding, not only of acid deposition in particular, but of atmospheric chemistry in general.

We are developing methods to apply our tunable diode laser absorption spectrometer to measure hydrogen peroxide. The spectrometer combines the high resolution of a tunable diode with the sensitivity provided by a long-path White cell. It should be highly specific to hydrogen peroxide without interferences from other gases.

Laboratory measurements with this system have shown detection limits of 1 to 2 ppbv. Although such detection limits would be adequate for polluted air improvements would be desirable for clean air measurements. We are developing computer data acquisition and analysis techniques to lower the detection limits. With the current version of this system the detection limit is better than 1 ppbv.

Field measurements also require suitable calibration and sampling techniques. We have developed a permeation device capable of delivering a flow rate of peroxide in the desired range which remains constant for periods of a week or more. A photometric analytical procedure has been perfected for determining the concentration of the calibration gas.

Tests have revealed that metals and epoxy glues remove peroxide from the gas stream while translucent Teflon, glass and the calcium fluoride windows do not. The sampling system is being constructed of these inert materials.

Hydrogen peroxide is believed to be the most important oxidant which converts S(IV) to S(VI) in the atmosphere. The processes are believed to be :

$SO_2(g) + H_2O = SO_2.H_2O(aq)$ (1)

$SO_2.H_2O(aq) = H^+ + HSO_3^-$ (2)

$HSO_3^- + H_2O_2(aq) \rightarrow SO_4^{2-} + H^+ + H_2O$ (3).

H_2O_2 is believed to be formed primarily from the recombination of HO_2 radicals:

$HO_2 + HO_2 \rightarrow H_2O_2$ (4)

During the daytime H_2O_2 also produces HO radicals by photolysis:

$H_2O_2 + hv(<340\text{ nm}) \rightarrow 2\ HO$

It therefore acts to convert HO_2 radicals to HO radicals. The HO radical plays a central role in the chemistry of photoxidants and acid deposition.

For these reasons, measurement of H_2O_2 in the atmosphere has received top priority. To date no measurement technique for this species has been demonstrated.

Infrared absorption presents an attractive opportunity for such measurements. Hydrogen peroxide has a well developed infrared absorption spectrum in the 8 u region. Very high resolution is required, however, to prevent interferences from other atmospheric constituents, particularly water vapour,N_2O and CO_2 which also absorb in this spectral region. The necessary resolution is available with the tunable diode laser absorption spectrometer (TDLAS) which we have developed.

The TDLAS has a number of advantages for atmospheric measurements. It is a passive technique with a response time of less than 1 minute, permitting in situ, real time measurments.

Because of its high resolution it provides positive identification of the target species with no interferences from other atmospheric species. By utilizing the long path length of a White cell it is able to detect most molecules in the fractional ppbv level.

A mobile TDLAS system has been constructed and used for field measurements of NO, NO_2 and HNO_3 (Schiff et al 1983). A schematic of this system is shown in Fig.1. The air is sampled through an all teflon inlet line into the White cell. A restriction at the inlet and a servo valve at the outlet maintain a constant air flow through the White cell. They also maintain the pressure in the cell at 25 Torr which reduces the pressure broadening of the line to an extent that makes interferences by other gases highly unlikely.

Calibration gases are added at known concentrations at the sampling inlet. Any losses that may occur in the sample line or in the White cell are compensated by this procedure.

The frequency at which a diode will lase depends on its temperature and the current passing through it. These diode typically operate somewhere in the 20 to 60 K range. A Helium cryocooler and a small heater maintain the appropriate temperature. The frequency of the diode can be scanned over a range of about 0.5 wavenumbers by changing the current passing through it. The laser beam is focussed into the White cell where it traverses a path length of 24 m. It is then focussed onto a HgCdTe detector. For initial set-up and spectroscopy a 10 cm cell containing the target gas at high concentration can be placed in the beam. The He-Ne laser is used initially to align the optics.

The spectrometer can be operated either in an amplitude modulated (AM) or a frequency modulated (FM) mode. In the AM mode the beam is chopped mechanically and the transmitted power detected at the chopping frequency. However, since the observed absorption features represent small differences between large power signals this method is limited to absorptions of about 1%.

To overcome this difficulty the spectrometer is operated in the FM mode. A 2 kHz sine wave is applied to the laser current, producing a frequency-modulated laser output. Detection is made at twice this frequency. A comparison of the the signals obtained is exemplified in Fig.2. Since only changes in laser power are detected a great deal of the noise is eliminated. Absorptions as low as 10^{-5} are detectable which, for a 24 m path length correspond to a detection limit in the fractional ppbv range. However, since information about the initial power is lost calibration is required to relate the signal to concentration.

The application of this technique to the measurement of a particular atmospheric species has three components: (1) The selection of the best rotational-vibrational line. The choice is usually a compromise between line strength and operating characteristics of the diode. (2) Development of a suitable calibration procedure. (3) Development of a suitable sampling procedure.

Selection of Spectral Line

Three laser diodes were tested from which one was selected. It operated in a single mode that could be scanned across two relatively strong H_2O_2 absorption lines at 1264.59 and 1264.62 cm^{-1}. The integrated line strengths of these features are 0.4 and 1.8×10^{-20} cm^{-1} respectively. The stronger line has 60% of the linestrength of the strongest line in the H_2O_2 spectrum which was unavailable with this diode. By comparison with our work on the nitrogen oxides this linestrength implies a detection limit for H_2O_2 of about 1.5 ppbv.

Figure 3 shows the FM signal for air containing 7 ppbv of H_2O_2 at the 2 selected wavelengths. Scans across these lines, with and without added peroxide are shown along with the limiting beam noise when the laser is held at a fixed wavelength. The detection limit is better than 2 ppbv as anticipated.

Calibration Procedure

Our philosophy of calibrating the instrument by adding a known concentration of the target gas at the air inlet requires a stable source of that gas in the ppmv range. Mixtures in this concentration range in steel or aluminum cylinders can be used for stable, non-polar gases such as NO, but are not suitable for H_2O_2.

We have successfully used permeation devices for the polar gases NO_2 and HNO_3. These devices are based on permeation from a high concentration source through a solid interface into a carrier gas stream. If the temperature of the device is carefully controlled the permeation rate remains constant for long periods of time. Liquids are generally used to provide the high concentration within the device and Teflon is the favoured material for the solid interface. Because of the low vapour pressure of even a 90% H_2O_2 solution the permeation rate through Teflon was too low for our purposes.

Higher permeation rates were achieved with low density polyethylene tubing. To prevent decomposition of H_2O_2 by impurities on the surface of the tubing it was cleaned with a hot solution of 50% H_2O_2 and sulfuric acid. A 4 m coil of this tubing, 0.32 mm O.D., 0.16 mm I.D. is immersed in a 90% H_2O_2 solution. At a temperature of 22 C and with a carrier gas flow of 20 standard cm^3 min^{-1} a concentration of 19.6 ppmv was obtained which remained stable for more than 1 week. This device therefore appears to be very suitable for our purposes.

To serve as a calibration source an analytical technique is required to provide accurate values for the permeation rate. The technique used is based on the colour change which occurs when H_2O_2 complexes with a solution of Ti(IV) (Pilz and Johann, 1974).

H_2O_2 is very soluble in water, having a Henry's Law constant of 10^5 molar atm^{-1} (Martin and Damschen, 1981). Because the complexing reaction with Ti(IV) removes aqueous H_2O_2 gaseous H_2O_2 can be effectively scavenged by this solution.

Interferences can occur when attempts are made to absorb H_2O_2 from real air. For example, the presence of SO_2 in the air can destroy H_2O_2 by the reaction sequence (1) - (3). By contrast, bubbling air containing O_3 through water has been shown to produce aqueous H_2O_2. The analytical technique is therefore not suitable for measuring H_2O_2 in real air but is suitable for analysing the gas from the permeation device which uses pure N_2 as the carrier gas.

Aqueous H_2O_2 reacts quantitatively with Ti(IV) to form a complex which is stable at pH <1. The nature of the complex is uncertain but the evidence is overwhelming that it contains a single peroxide molecule. Thus, in the presence of excess Ti(IV) quantitative conversion of H_2O_2 to the complex form can be assumed. The Ti(IV) solution is colourless, having an absorption in the u.v. with a maximum below 330 nm. The peroxide complex is red-orange with a maximum absorption at 410-415 nm. The maximum absorption coefficent is 735 M^{-1} with no contribution from the Ti(IV) solution itself. Optical absorption at 410 nm can therefore be used to determine the peroxide complex and consequently the gaseous H_2O_2 concentration.

The analytical procedure used is as follows. 11.6 ml of pure $TiCl_4$ is dissolved in 50 ml of concentrated (37%, 12M) HCl. Care must be taken at this stage since $TiCl_4$ reacts vigorously with moisture to generate HCl and a great deal of heat. The resulting solution is bright yellow and keeps indefinitely.

This solution is diluted ten-fold with distilled water to produce a solution (B) which is colourless and cannot be stored for more than a few days since it slowly decomposes with precipitation of TiO_2.

One ml of solution B is diluted with 20 ml of 3.7% (1.2 M) HCl. This solution is placed in a microimpinger and the calibrating gas stream bubbled through it until yellow coloration is observed. The sample is removed, the impinger washed with 3.7% HCl to dilute the solution to 50 ml. The absorbance is measured at 410 nm using a Bausch and Lomb visible/uv spectrophotometer. From this measurement, the known absorption coefficient of the complex and the flow rate of the gas through the impinger the concentration of H_2O_2 is determined.

Sampling Procedure

Since H_2O_2 is reactive and decomposes readily, studies were made of various materials to test their suitability for use in the sampling system. These tests were performed by inserting the materials between our H_2O_2 source and the Ti(IV) solution. Metals such as stainless steel were found to remove significant amounts of the peroxide from the gas stream and do not appear to be conditioned. All epoxy resins and Torr seal react to remove the peroxide from the gas stream completely. Glass, translucent Teflon and dry calcium fluoride (used for cell windows) do not remove the peroxide from the gas stream provided that they are preconditioned for a few minutes with the gas mixture. The only sealant we have found suitable for optical windows is Apiezon W hydrocarbon wax.

The calibration and sampling system is currently being built from these inert materials and tests will be conducted to determine the conditioning time of this system to changes in H_2O_2 concentrations. This will determine the response time of this instrument for this gas.

Minimum Detection Limit

The detection limit of better than 2 ppbv mentioned earlier is acceptable for measurements in polluted air where the H_2O_2 concentrations higher than 10 ppbv have been reported (Kok et al, 1978). Concentrations in rural air, however, are likely to be below this detection limit and improvements must be made for regional scale measurements.

The most promising method for improving the detection limit appears to be by data processing. Much of the noise observed in the analog detection method shown in Figure 3 is frequency dependent and should be amenable to data averaging procedures to remove the background spectrum. Considerable efforts have been expended in creating software for such data handling. Figure 4 shows some preliminary results where curve C is obtained by subtracting by microcomputer the background curve B from the curve A obtained with a gas mixture containing 3.9 ppbv of H_2O_2. The detection limit has been greatly improved by this procedure to well below 1 ppbv. Further improvements are likely from our continuing software development.

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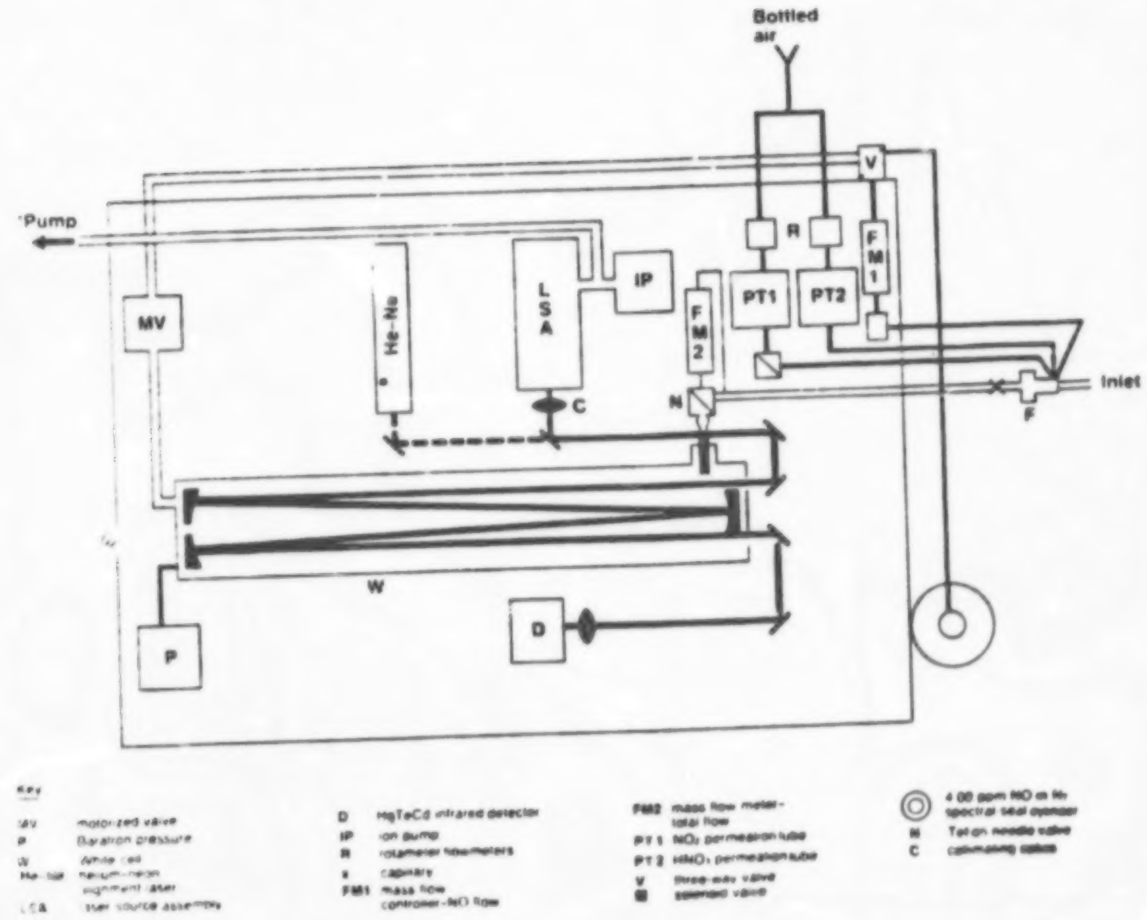
Bottled air
V
Pump
R
FM1
IP
PT1
PT2
MV
He-Ne
LSA
FM2
N
C
Inlet
F
W
D
P
Key
MV motorized valve
P Baratron pressure
W White cell
He-Ne helium-neon alignment laser
LSA laser source assembly
D HgTeCd infrared detector
IP ion pump
R rotameter flowmeters
x capillary
FM1 mass flow controller-NO flow
FM2 mass flow meter-total flow
PT 1 NO₂ permeation tube
PT 2 HNO₃ permeation tube
V three-way valve
solenoid valve
4.00 ppm NO in N₂ spectral seal cylinder
N Teflon needle valve
C collimating optics

FIGURE I. MOBILE TDLAS SYSTEM

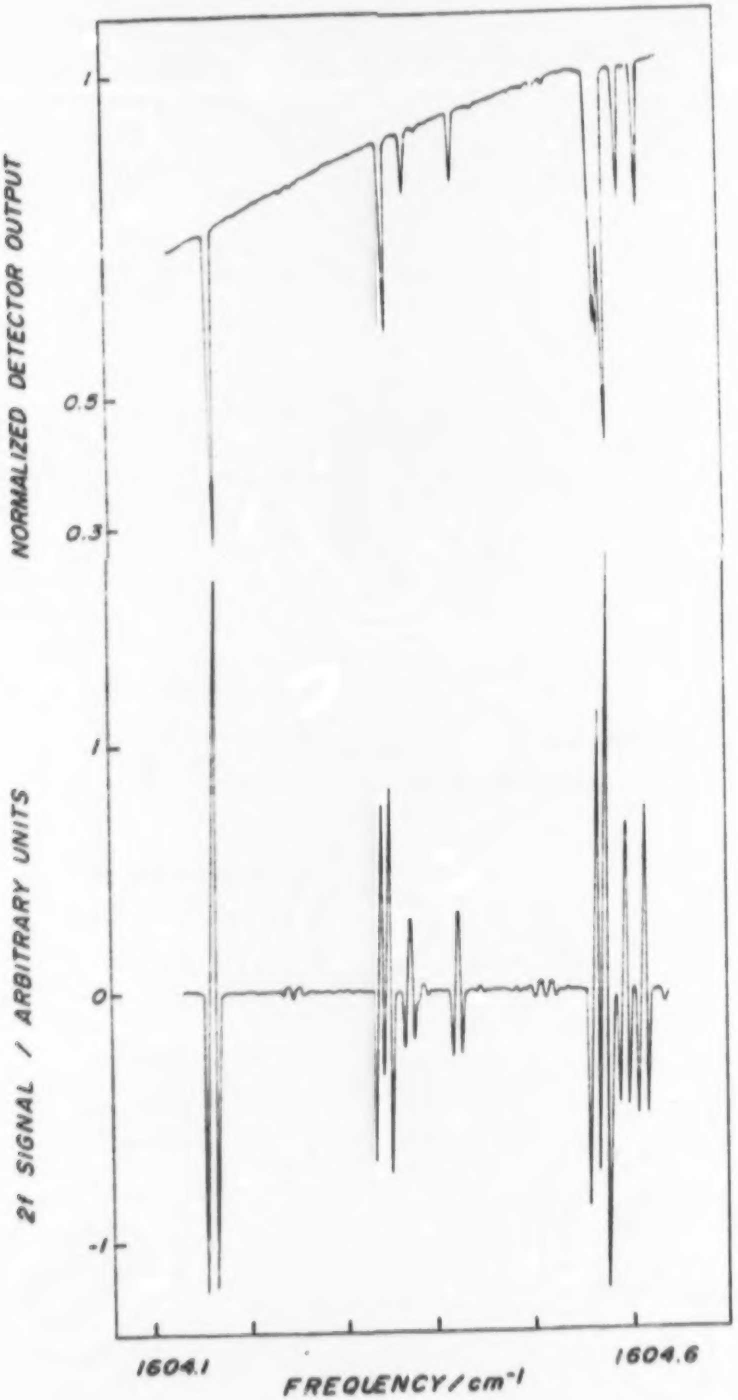

FIGURE 2. AM (TOP) AND FM (BOTTOM) ABSORPTION SPECTRA OF NO_2.

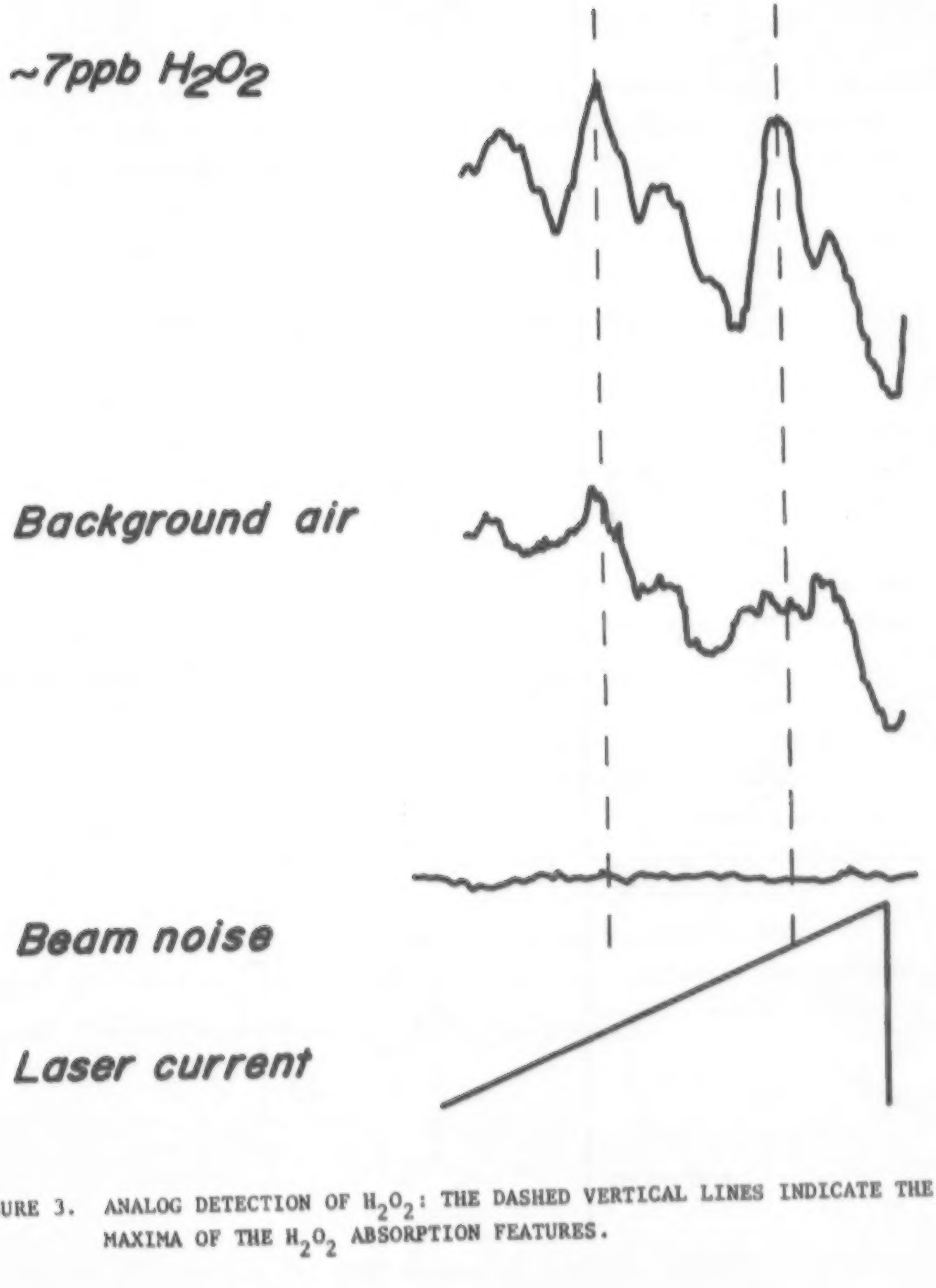

FIGURE 3. ANALOG DETECTION OF H_2O_2: THE DASHED VERTICAL LINES INDICATE THE MAXIMA OF THE H_2O_2 ABSORPTION FEATURES.

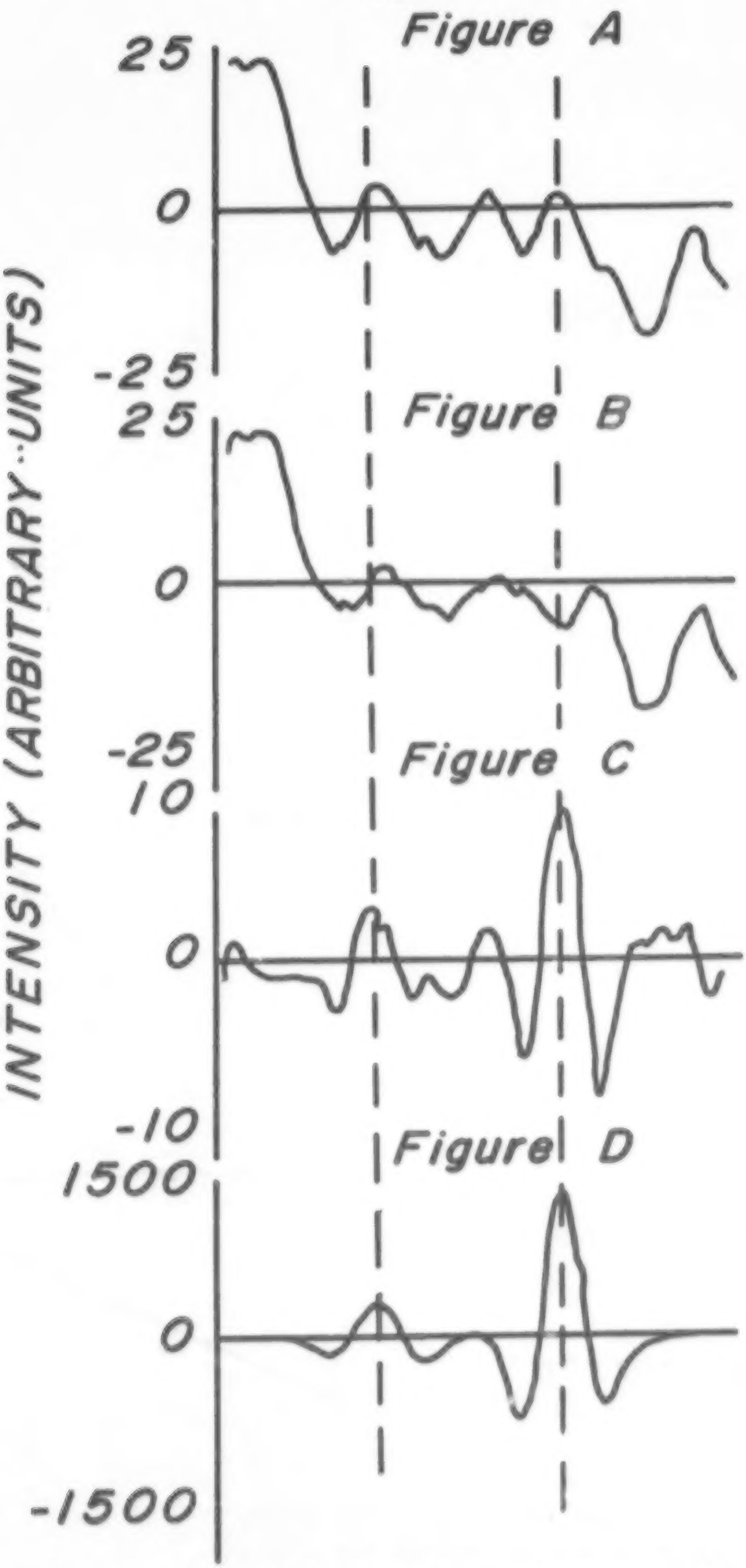

FIGURE 4. COMPUTER ASSISTED H_2O_2 DETECTION. A: 3.9 ppb H_2O_2 IN AIR; B: BACKGROUND. C: A-B; D: REFERENCE SHOWING THE H_2O_2 ABSORPTIONS.

The Dispersal of Airborne Particulates on a Short and Long Term Basis

J. D. MacArthur and G. R. [illegible]lmer
Queen's University, Kingston, Ontario. K7L 3N6

ABSTRACT

The environment receives many substances from anthropogenic and natural sources. In order to understand their extent and effect, many measurements of various types are necessary. Proton induced X-ray emission has been used to measure the element concentrations at the ng/m^3 level in air particulates collected by means of an air streak filter which provides temporal records on a short time scale of about an hour. Also concentrations have been measured at the ppm level in tree rings which provide a temporal record over many years.

Two species of pine trees at each of two locations, one near and downwind and the other relatively distant and upwind from Sudbury have been examined to determine the effect of the smelters in that city. No distinctive differences attributed to the installation of the high INCO stack in 1973 were observed. However, the cores of trees taken from near Sudbury showed increased concentrations for most of the elements, S, Cl, K, Ca, Mn, and Fe. When scans of the concentrations were measured with a beam of 25 μm, variations in the levels of several of the elements were observed during the growing season.

Streak filters were taken during a three week sampling period last winter at ground level some 50 m from a busy intersection in Kingston. Large variations in the levels of several elements were detected and these correlated highly with vehicular traffic. This was especially true for lead. Furthermore, Na, Cl and Ca levels were strongly correlated, suggesting a common origin which might be the sanding mixture since the residual fine road dust showed high levels of these elements. Daily averages for the concentrations observed inside and outside a school at the site were also measured.

That the quality of life depends significantly on the quality of the environment is becoming well recognized. Unfortunately, the environment is a dump, intentionally and unintentionally, for many wastes produced by society. In order to assess the extent of these wastes it is necessary to measure the concentrations of chemicals and elements as well as their movement through the environment. We have used proton induced X-ray emission (PIXE) and proton induced gamma ray emission to measure the element concentrations (1) tree rings in order to observe trends over many years and (2) of air particulates in order to observe the dispersal of automobile exhaust from a busy intersection.

AIR PARTICULATE PROJECT

Introduction

Air contains particulates, mostly between 0.1 and 10 μm in radius held in suspension by Brownian motion. The lower and upper limits are determined by coagulation and gravitational settling respectively. Understanding the source, transportation and removal mechanisms for these particulates is important since they may contain harmful substances, impair visibility and modify climates (Johansson et al., 1974)

Urban aerosols possess a bimodal mass distribution with gaussian peaks centred at 0.5 and 5.0 μm (Matsuo et al., 1978, Cahill et al., 1977, Whitby et al., 1972) which are characteristic of high-temperature combustion and natural sources respectively. Besides influencing the transportation of these aerosols their size is significant in determining where the particles are trapped in the human respiratory tract. The small particles, containing nearly all the toxic inorganic and organic materials, reach the pulmonary regions of the lungs (Natusch and Wallace, 1974).

A significant contribution to the smaller sized fraction of urban aerosols arises from automobile exhausts. Numerous studies of vehicle emissions have been conducted to characterize the size distribution (Martens et al., 1973; Paciga et al., 1975; Chan and Lawson, 1981), the chemical content (Habibi, 1973) and the dispersal (Chock, 1982; Bullen et al., 1982) of exhaust particulates. Considerable health hazards are associated with emitted lead salts resulting from the addition of tetraethyl lead to gasoline as an anti-knock agent. Lead affects the central nervous system causing learning and behavioral problems in young children (Marshall, 1982). Estimates show that 50% of the lead absorbed by the body (∿23 μg/day) is inhaled (Goldsmith and Friberg, 1976) with 30% of the lead in the blood originating from gasoline (McGinty, 1982). A direct correlation was observed between the drop in blood lead levels and the 30% decrease in lead used for gasoline in the U.S. between 1976 and 1980 (NHANES, 1976-1980).

In an attempt to assess the contribution which proton induced X-ray emission could make to a study of air particulates, a streak air filtering system was used to sample air near a busy intersection in Kingston. In addition, its performance has been compared to a dichotomous sampler in a joint study with the Kingston office of the Ontario Ministry of the Environment.

Experimentation

The air streak filtering system (Jensen and Nelson, 1974; Barfoot et al., 1979) was housed in a rectangular box 0.6x0.2x0.2 m^3 in size, open on the long sides to admit air through a coarse mesh. This box was mounted, 1.5 m from ground level and air samples were collected at a site approximately 100 m SSW of a busy intersection (38,000 cars/day) and approximately 3 m from a two story school for five weeks in Dec. 82 and Jan. 83. The air streaks were collected on 0.4 μm Nucleopore moving at 1.0 mm/hr past a 2x2 mm^2 nozzle through which air was drawn at 0.15 l/min. This flow was observed to be constant within 10% for the duration of the sampling time. A simple calculation suggests that with this flow rate, all particles less than ∿30 μm would be collected.

When it became clear that visible deposits were being collected at times of high traffic density, the study was expanded by placing a second sampler inside the school in order to compare the air inside and outside the building. Since considerably less material was expected inside the school (an assumption which proved to be false), only daily samples were collected on 0.4 μm Nucleopore rather than on extended streaks.

Many studies (Watson et al., 1981) have shown that different filtering devices produce particulate samples with different size characteristics. Since the Ontario Ministry of the Environment has chosen the dichotomous sampler, which collects fine and coarse fractions separately, to conduct a preliminary survey of the air quality throughout Ontario, the streak and the dichotomous samplers were run simultaneously in the same place so that a comparison could be made. The site chosen was atop the four-story Ontario Government Building in Kingston. During this comparison, the filters in the dichotomous sampler were changed every two hours over a twenty four hour period to match the two-hour resolution of the streak sampler.

All the filters were analyzed for the elements present using an external beam of protons from the Queen's University Van de Graaff accelerator and the technique of proton induced X-ray emission (PIXE). In this technique, protons of a few MeV energy bombard the sample dislodging electrons from the constituent atoms. These atoms emit X-rays which are characteristic of the atom. The X-rays are detected by a Si(Li) detector which produces a spectrum of the number of times an X-ray with a specific energy is observed. A typical spectrum from a spot on an air streak deposit is shown in Fig. 1.

The deposits from the air samplers are thin in the sense that the protons pass through the deposit losing relatively little energy ($\sim$100 keV). Hence the cross section for exciting the atoms is essentially constant and the self absorption of the X-rays is quite small. The number of X-rays coming from a specific element, x, may be written;

$$N_x = N_p \, n_x \, \Delta t \, \sigma x(E) \, \omega x \, \varepsilon_x \qquad \text{A}$$

where N_p is the number of incident protons,
$\sigma_x(E)$ is the cross section for dislodging an electron from element, x, when the energy of the proton is E,
ω_x is the probability that an X-ray is emitted when the atom relaxes,
ε_x is the detection efficiency for the detection of the X-ray produced,
n_x is the number density of the element,
and Δt is the sample thickness.

The quantities N_p, $\sigma_x(E)$, ω_x and ε_x are determined experimentally by placing a layer of an element of known thickness in the beam at the same spot as the sample and measuring N_x. Hence the system is calibrated and $n_x \Delta t$, the areal density, for each element of the deposit is determined. Then, since the total area of the deposit as well as the flow rate is known, the amount of the element in the air can be determined.

Results

Fig. 2 is a photograph of a one-week streak with the Pb levels, precipitation and wind direction [illegible] on the same time scale. Strong correlation between the Pb levels and the dark deposits is evident. These deposits are in turn related to the periods of heavy traffic at the intersection, except when the winds were not blowing towards the site from the intersection. Precipitation does not seem to significantly affect the lead levels. In another test, measurements were made on the amount of lead in the road dust with a null result. Consequently, the automobile exhausts must be the source of this pollutant.

Time scans for peak areas of several other elements are shown in Fig. 3. Certain scans, e.g. S, appear unrelated to all other elements implying that S has a unique source. However, correlation is obvious for some of the elements, e.g. Cl, Ca and Na, thereby suggesting a common source. Fig. 4 is a scatter plot for Ca and Cl. These two elements are seen to be interdependent with a correlation coefficient of 0.88. Moreover, Cl, Ca and Na have high concentrations on Sat and Wed during rush hours, indicating their common origin is dependent on traffic. It is hypothesized that this source is road salt. If $CaCl_2$ and NaCl were in equal proportions in the salt, the atomic ratio of Ca:Cl should be 1:3. Table I lists the concentration values for the major elements detected in Kingston air and compares these levels with those measured in other locations. From the Ca and Cl levels it can be determined that the measured atomic ratio is about 2.7, not inconsistent with the above assumption for the salt mixture.

Table II shows the results of the study in which 24-hour samples of the air particulates inside and outside the school were studied. For the two days studied, Table II shows that the concentrations of most elements were comparable inside and outside the school. The Nucleopore used in this study was free of Br; hence the Br/Pb ratio in Table II may be used to conclude that the source of the lead is gasoline since that ratio is within the range observed for gasoline exhaust (Paciga et el., 1975).

A comparison of the results of the streak and dichotomous samplers is presented in Table III. The concentrations are averages for the set of two-hour samples taken. The results for the individual two-hour samples are not presented because they are the same as for the average except that they are of lower statistical accuracy. An examination of the numbers reveals that, in general, the streak values approximate those for the fine fraction of the dichotomous sampler. The values for Ca and Al are somewhat higher indicating that some of the coarse fraction of the air particulates containing these elements is collected on the air streak deposit. Consequently the results from the streak sampler should be a good indicator of the air particulates which are likely to lodge in the lungs. Lead was not detected at this elevated site (<0.05 μg/m3). This seems surprising since the site near the busy intersection can be seen from this site and there are intervening roads and an adjacent shopping center where the traffic density is high. However, the streak sampler did reveal two instances of high lead concentrations (1.6 and 2.1 μg/m3), both in the middle of the night when the dichotomous sampler was not operating. Bromine was not detected at the same time which indicates that these high lead levels were not due to automobiles. Their cause remains unexplained.

Conclusions

An air streak sampler has been shown to be useful for observing changes in lead levels in air due to variations in traffic. Furthermore, the technique can monitor the level of other elements from other sources. The quantity of the air particulates inside and outside a building have been shown to be similar. However, the time variation of this correlation has yet to be established.

In comparison to a dichotomous sampler, it appears that the streak sampler collects the fraction of air particulates which appear in the fine stream of the dichotomous sampler. Consequently, streak samples can reveal time variations in the presence of air particulates that are likely to reach the lungs.

TREE RING PROJECT

Introduction

Many minor and trace elements have been observed in measurable quantities in trees (Meyer and Langway). Furthermore Hacskaylo et al. (1969), in studying the nutritional requirements, has indicated that other elements should be present in trees. Studies such as that of Thompson (1981) have shown that the presence of pollutants, due to a nearby smelter, will influence the width of tree rings. That variations in the elemental concentrations might record the pollution in the environment was suggested by Valkovic et al. (1979) and Lepp (1975).

A heartwood-sapwood differentiation in the concentration of chlorine was observed by Tout et al. (1977) using neutron activation analysis. Several other studies (Robitaille, 1981; Pillay, 1976; Baes III and Ragsdale, 1981) using values from every fifth year or five year averages have shown that the level of metals e.g. Pb, Cu and Zn, are elevated near smelters and roads carrying a high traffic density, indicating that pollution in the environment may be being recorded. However, large variations in the concentrations were observed suggesting that the process of element deposition in trees is complex. Consequently, the present study of the elements in tree rings was undertaken with the external proton beam from the Queen's microprobe (MacArthur et al., 1981) because it has a spacial resolution less than the width of tree rings.

Experimentation

Since variations in element concentrations in trees were being sought, cores were obtained from trees at several locations at various distances and directions from the nickel smelters in Sudbury. It is well known that this region shows the effects of pollution (LeBlanc et al., 1972). Consequently, it was felt that variations in levels of the different elements might be observed for trees from this region, especially since their environmental conditions were supposedly changed with the introduction of the high INCO stack in 1972. Two 1/4" radial cores were taken from each tree, one at stump height i.e. 0.5m from the ground and one at chest height i.e. 1.5m from the ground. These cores were mounted in teflon blocks and flat surfaces were prepared by filing. Pictures of these surfaces are shown in Fig. 5. This preparation requires care because surface roughness as small as 10 μm can produce differences in counting rates due to the absorption of the X-rays. This is especially true when the detectors do not view the surface normally. Microscopic examination of the surfaces indicated that the roughness was less than a few μm. These samples were then mounted on a microstage and moved past the proton beam in steps as small as 80 μm.

These tree ring samples are thick samples, unlike the air streak deposits because the protons stop in the wood. Consequently, to find the number of X-rays observed, expression A must be integrated over the range of the proton as it travels through the wood. The counting rate observed is

$$N_x = \epsilon_x \ \epsilon x \ N_p \int_0^T \sigma_x(E) \ n_x(t) \ a(t) \ dt$$

where a(t) is the absorption of the X-rays from the depth t

and T is the depth to which the proton penetrates before it stops. Protons and other charged particles lose energy at a known rate as they travel through matter i.e. $dE/dt = S(E)$ where S(E) is the stopping power of the material, in this case wood. as a function of proton energy, E. Therefore

$$N_x = \omega_x \ \epsilon_x \ N_p \ n_x \int_0^T \sigma_x(E) \ a(E) \ dE/S(E)$$

if n_x is assumed constant along the path of the proton.
Hence, the number of X-rays observed is independent of the thickness and density. The calibration of the system can be accomplished with substances of essentially the same basic composition as wood and which contain known quantities of elements likely to be found in wood. Since the sensitivity varies smoothly from one element to the next, the system does not have to be calibrated for every element expected.

The calibration was carried out with the following standards from the National Bureau of Standards - orchard leaves, pine needles and bovine liver. Two comments should be made about this calibration procedure. Firstly, the beam was moved on the sample in order to approximate the recommended sample size of 250-500 mg necessary for a reliable calibration using these standards. Secondly, it is necessary to assume that the concentrations of H, C and O and possibly N are similar in the standards and in the tree rings so that S(E) is similar in the two cases. This is not a stringent requirement since C, O and N have roughly the same stopping powers. Therefore, protons will penetrate to the same depth in samples with similar proportions of H, C, N and O, such as the NBS standards and the wood samples.

Results

Several findings about the usefulness of PIXE and tree rings in the study of the distribution of elements in time have emerged from this investigation.

Fig. 6 shows the results for two of the many scans in the radial direction observed to date. Both scans are for the element Ca, one being for a core from a red pine on Levesque St. in Sudbury and the other for a core from a white pine at Penage. The two detectors on opposite sides of the beam observed the same variations at the same position which would not be the case if roughness were masking one detector and then the other. Consequently, these variations together with the fact that the counting rates are related directly to the concentrations, as was pointed out earlier, means that variations in concentrations of the elements are being observed.

To investigate how localized these changes might be, two scans 0.5 mm apart were taken through the same section of the core. Very similar patterns were observed. Consequently, the observed variations are not localized on the scale of 100 μm but are distributed concentrically with the centre of the tree in a fashion similar to rings.

For the red pine, increases in Ca levels occur in the early spring each year. For the white pine, this correlation is not as evident. However, the ring pattern for this white pine was very indistinct. In fact, many of the marks on the figure indicate positions where very narrow lines of slightly different shade were observed. Consequently, whether all the rings are being observed or not is unclear. Other scans of cores from white pines showed very clear rings which were perfectly matched with the changes in the Ca level each growing season. Whether careful measurements of these changes can be used as a tree ring count is still uncertain. The figure shows scans for Ca only. Elements such as K and Mn showed essentially the same pattern as Ca while others such as Fe, Cu and S showed no pattern in which the variations were correlated with the seasons. On the other hand, metals often showed high, quite-localized concentrations. Visual observation of these regions of the core, never revealed any structure or speck which would account for these elevated levels.

Fig. 7 shows addition scans to show what little evidence has been found to indicate that there are long term trends in the levels of the elements. The dates indicated on the figure were determined by counting rings from the bark inward. Clearly if rings are missed, these dates are in error. Nevertheless, the commissioning of the high stack in 1972 did not immediately influence the deposition of these elements. Furthermore, the concentrations of Ca and Mn have fallen with time in both species of trees while the level of Fe has remained constant.

Table IV presents average concentrations for the elements observed routinely in the two types of trees. Trees both distant and near the smelters have been analyzed. Although there is an indication that trees nearby have higher concentrations than those further away, such a conclusion can only be drawn after considerably more samples have been analyzed.

Conclusions

It has been shown that PIXE can reveal the presence of many elements at quite low levels (a few ppm) in tree rings. Variations in the concentrations of some of these elements, particularly Ca, Mn and K, occur throughout the annual growing period and these variations can possibly be used to measure the annual growth pattern.

The influence of a major, point source of pollution on the level of trace and minor elements in trees has probably been established. The level of these elements did not change dramatically or suddenly when efforts were made to reduce the amount of pollution from this point source although the level of some of the elements has decreased since the change was effected.

TABLE I

Concentration (μg/m3) of the Elements in the Air

	Site				
	Kingston		Rural U.K.	Mexico City	St. Louis
Element	Maximum	Mean	Mean	Mean	Mean
S	0.7	0.1		8.	4.4
Cl	5.	1.	2.	1.	0.08
Ca	2.2	0.5	0.6		0.11
Fe	0.5	0.08	0.13	3.6	0.22
Pb	0.7	0.2	0.04	2.5	0.72

TABLE II

Average daily concentrations (μg/m3) found inside and outside the school

	Monday, Jan. 31		Tuesday, Feb. 1	
Element	Inside	Outside	Inside	Outside
S	6.0	1.8	1.3	1.2
Ca	0.41	1.3	0.91	5.7
Mn	0.038	0.050	0.047	0.13
Fe	0.13	0.25	0.20	1.0
Zn	0.059	0.043	0.054	0.078
Br	0.037	0.015	0.060	0.060
Pb	0.27	0.17	0.34	0.57

TABLE III

Element concentrations (μg/m3) in air compared for samples collected with the air streak sampler, with the fine and coarse stream of the dichotomous sampler

Element	Streak	Fine	Coarse
Al	0.83	0.25	1.7
Si	0.31	0.40	7.4
S	0.25	0.27	<0.05
Cl	0.40	0.50	8.4
Ca	1.70	0.23	7.1
Fe	0.20	0.15	2.8
Cu	0.03	<0.005	<0.01
Zn	0.012	0.05	0.07

TABLE IV

Concentration of elements in ppm for two species of trees comparing measurements taken on trees growing 8 km and 30 km from the smelter in Sudbury

Element	White Pine		Red Pine	
	Near	Far	Near	Far
S	608	615	200	80
Cl	210	139	171	139
K	730	500	810	550
Ca	1200	650	1520	990
Mn	50	25	100	250
Fe	100	65	60	30
Cu	17	14	35	19
Zn	26	22	35	29

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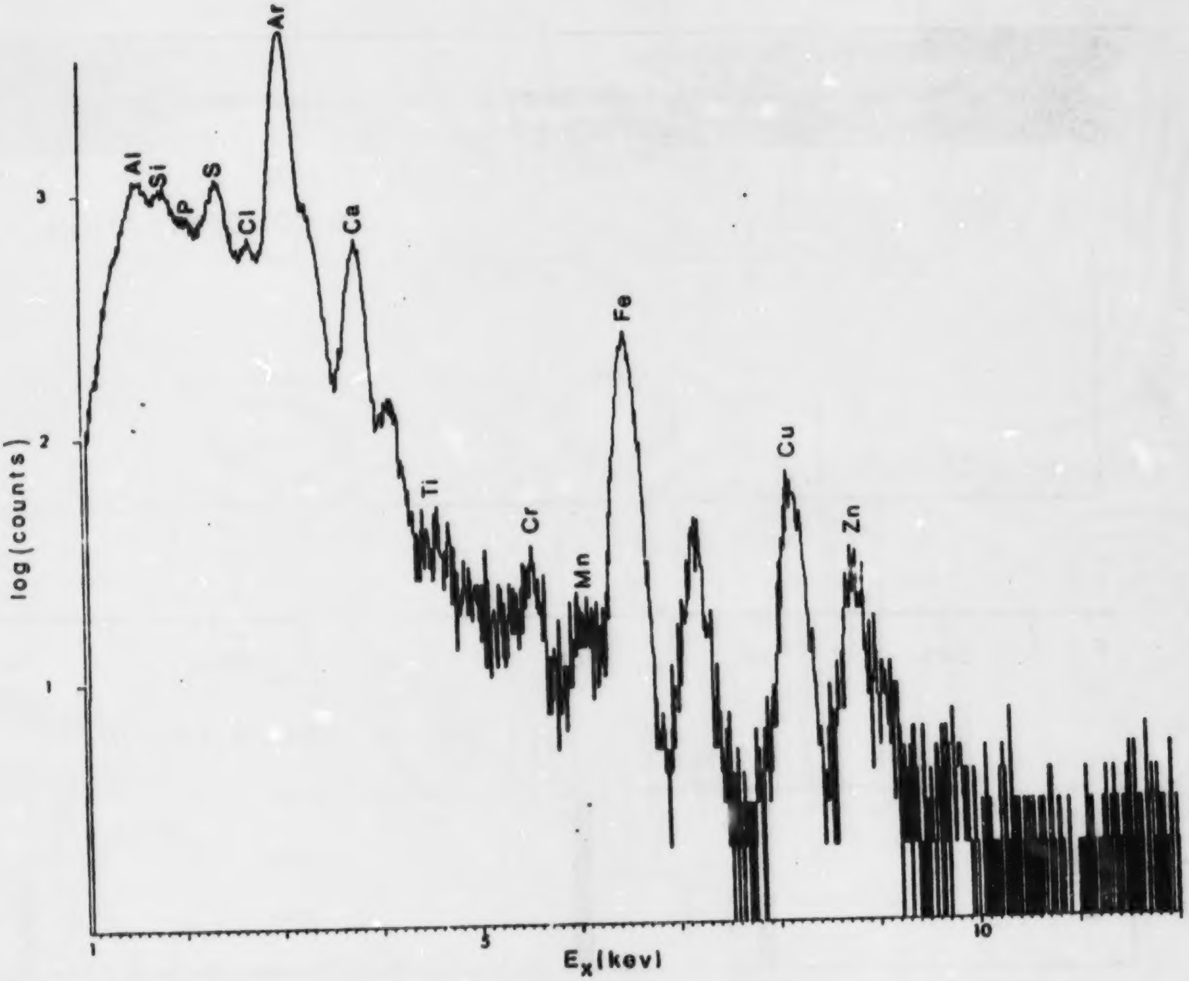


FIG. 1

An X-ray spectrum from an arbitrary spot on a streak sample taken on the roof of the Ontario Government Building in Kingston. The sample was excited in air with a proton beam of 2.5 MeV, hence the presence of a strong Ar line. Unlike spectra observed from the streaks obtained at ground level, this spectrum shows no sign of Pb at an energy of 10.5 keV.

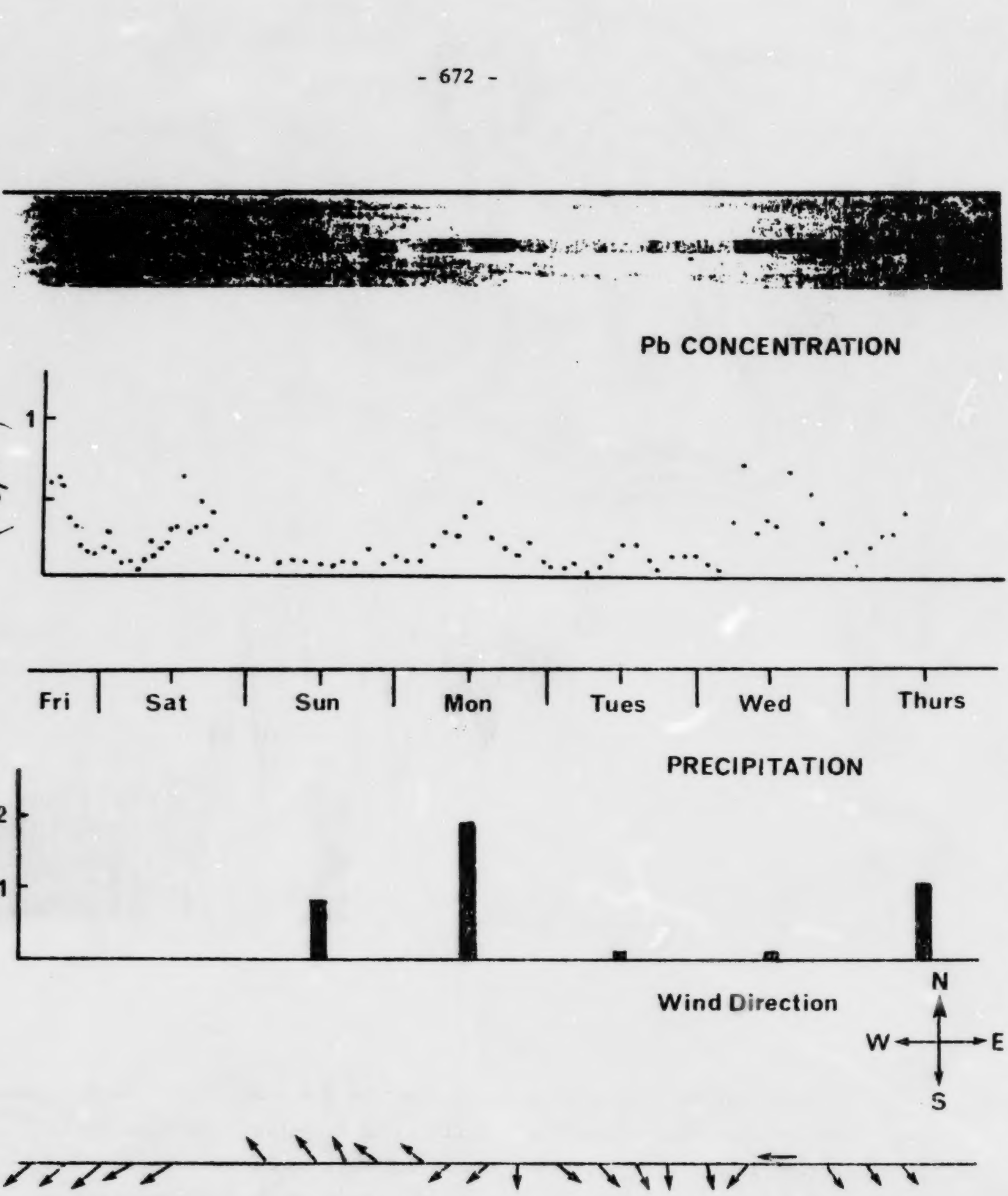


FIG. 2

A photograph of an actual streak together with meteorological data and a time profile of the lead concentration which is correlated with high traffic densities.

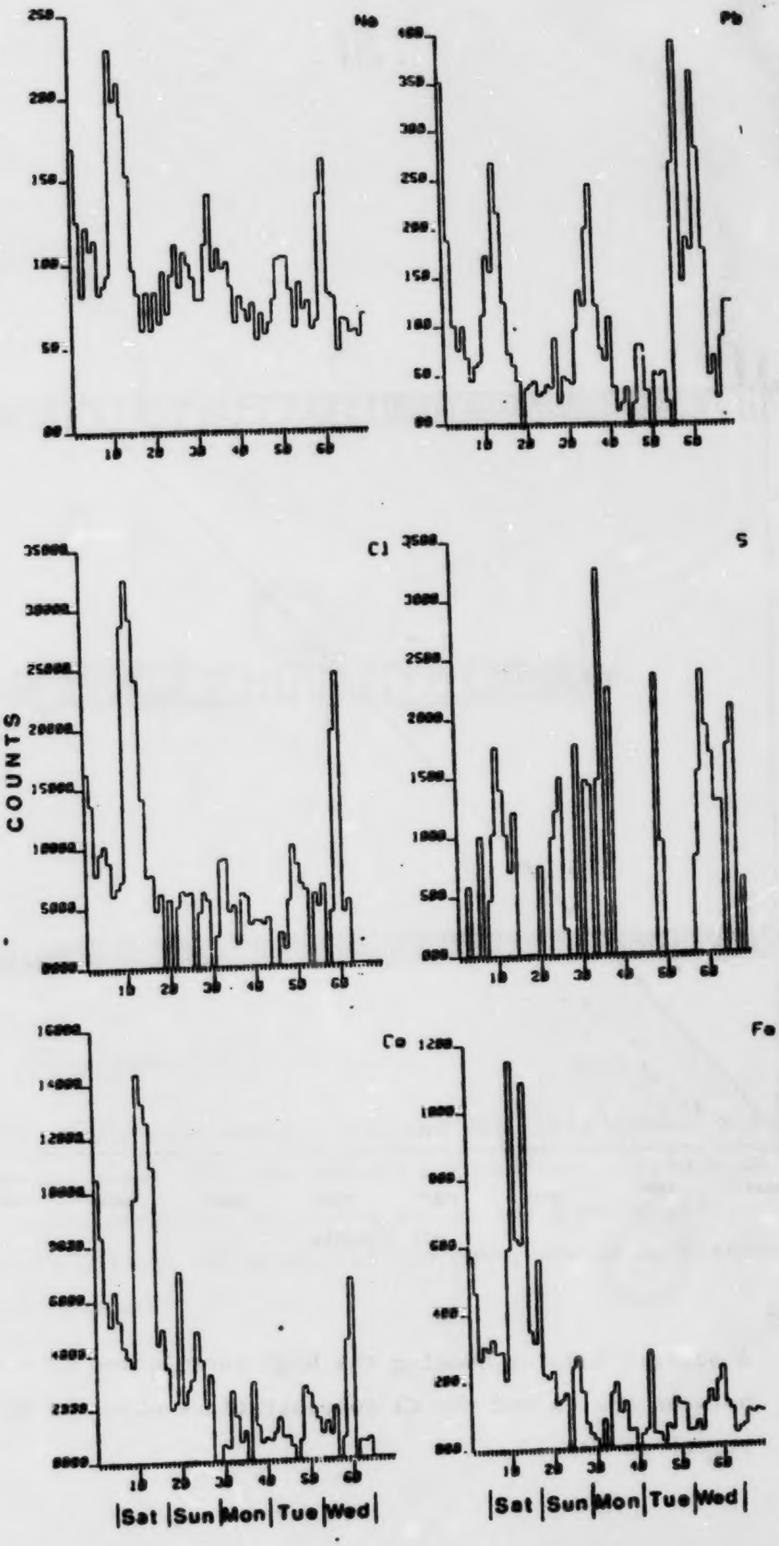


FIG. 3

Time scans for the elements detected in the particulate matter trapped on the air filter of Fig. 2. Each bar represents an average over a two-hour period.

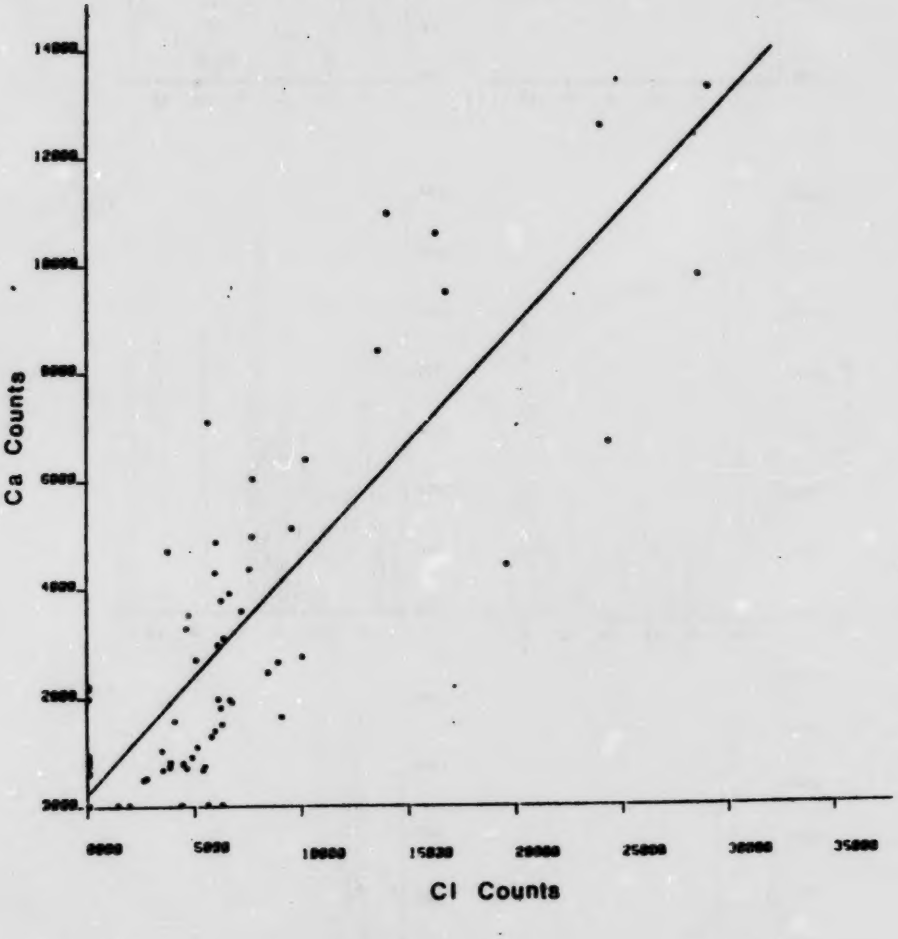


FIG. 4

A scatter diagram showing the high correlation (r = 0.88) between the Ca and the Cl concentrations observed in the streak of Fig. 2.

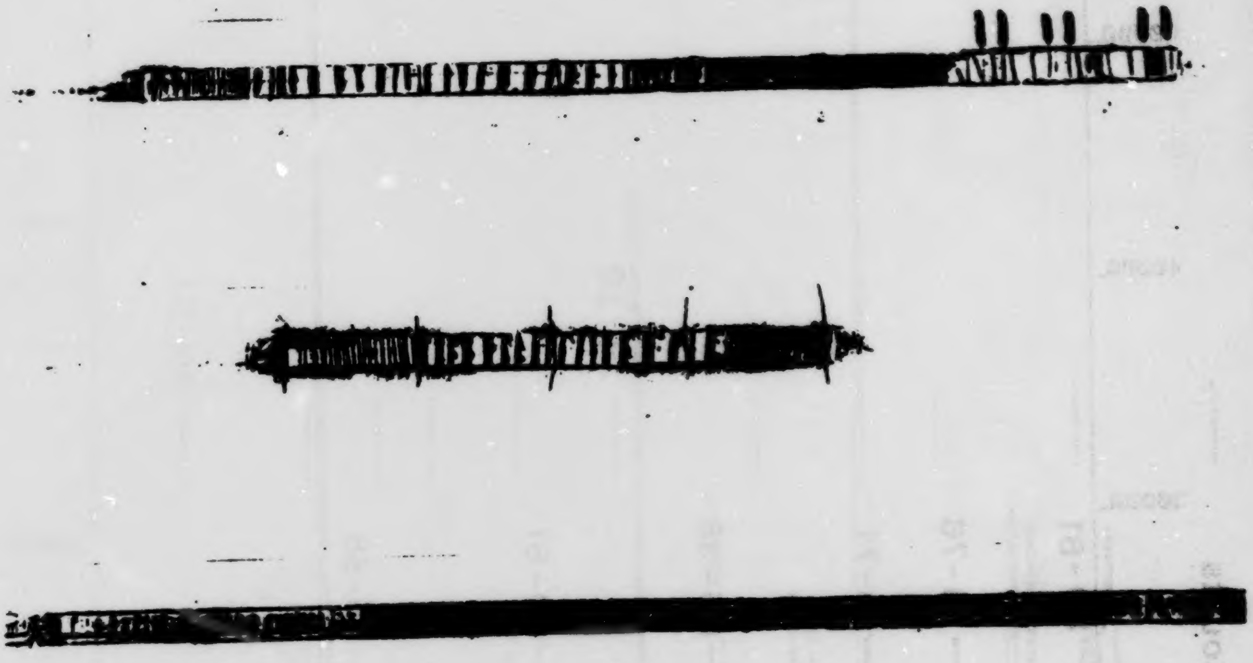


FIG. 5

Photographs of the core samples analyzed for their element concentrations with a 25 μm proton beam. The top two photographs are for red pine where the tree rings are quite distinct; the third one is for a white pine. The lines, approximately through the middle of the core, are an indication of where the beam hit the samples.

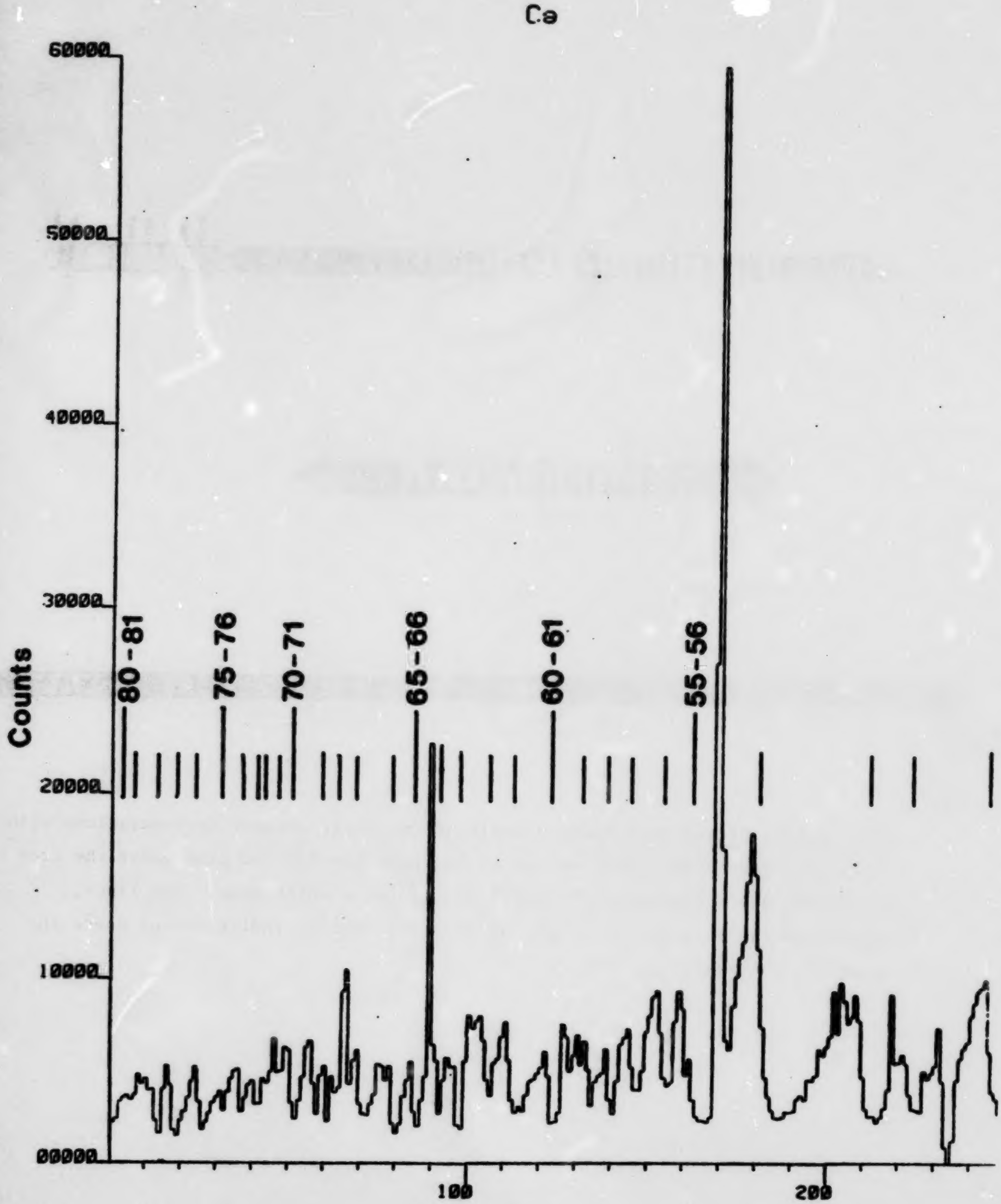


FIG. 6a

Time scans of the Ca counts in a sample of red pine. The lines added to the figure indicate the position of the dark rings observed visually. There is a distinct pattern in which the Ca counts rise in the spring.

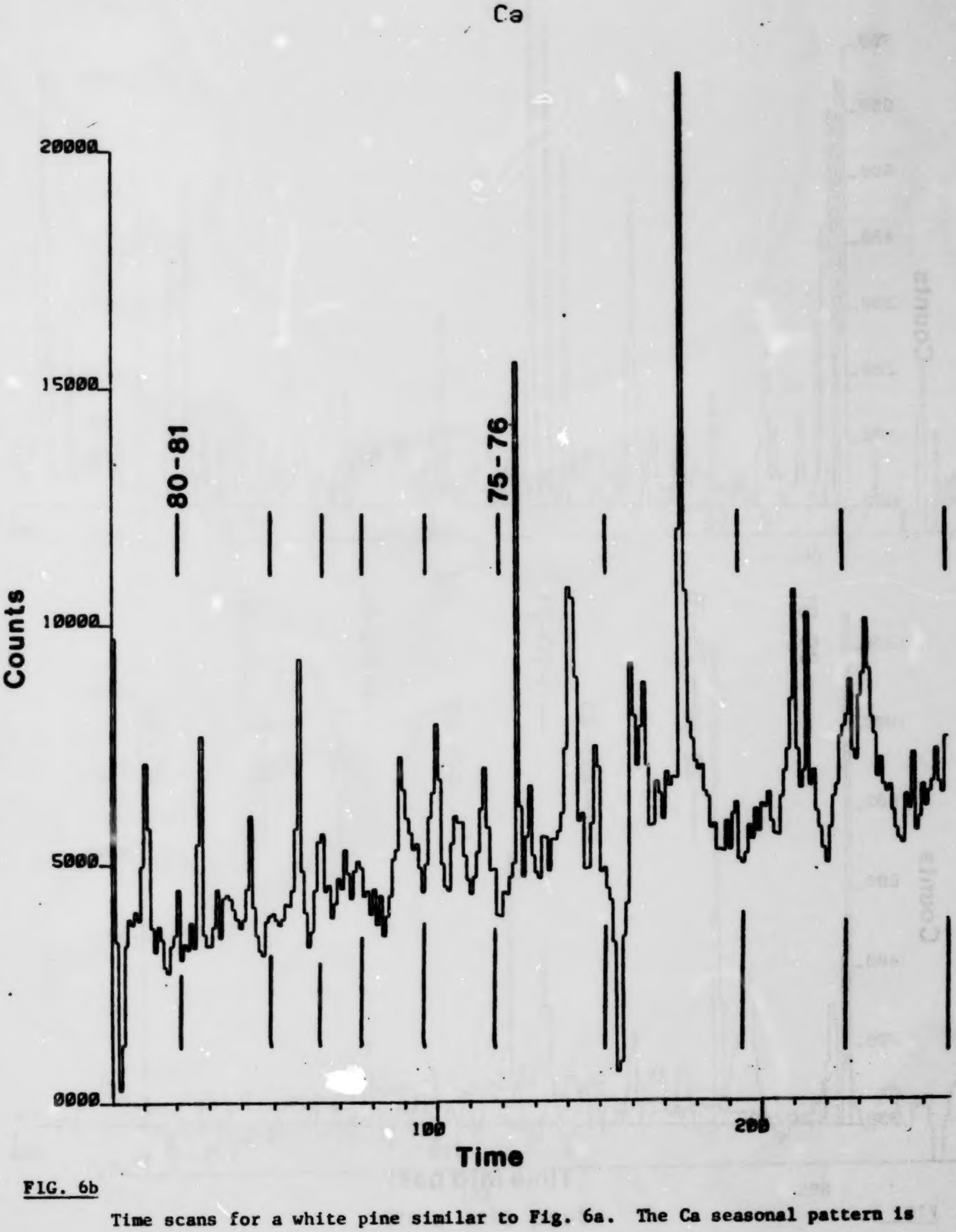


FIG. 6b

Time scans for a white pine similar to Fig. 6a. The Ca seasonal pattern is not obvious.

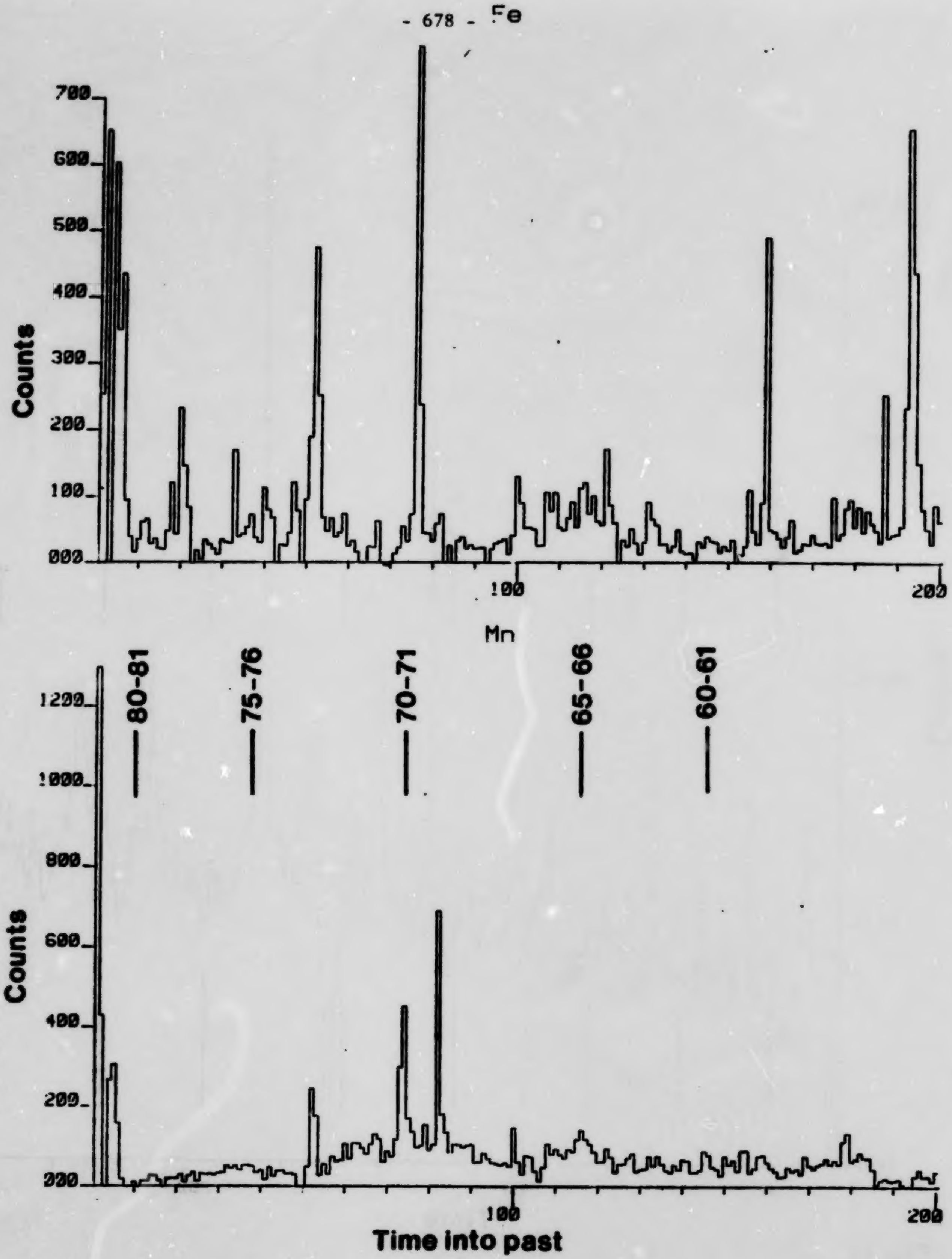


FIG. 7

Time scans for Fe and Mn counts in samples of white pine(a) and red pine(b) taken near the smelters in Sudbury. After 1973, the concentrations of Ca and Mn decrease while the Fe levels remain constant.

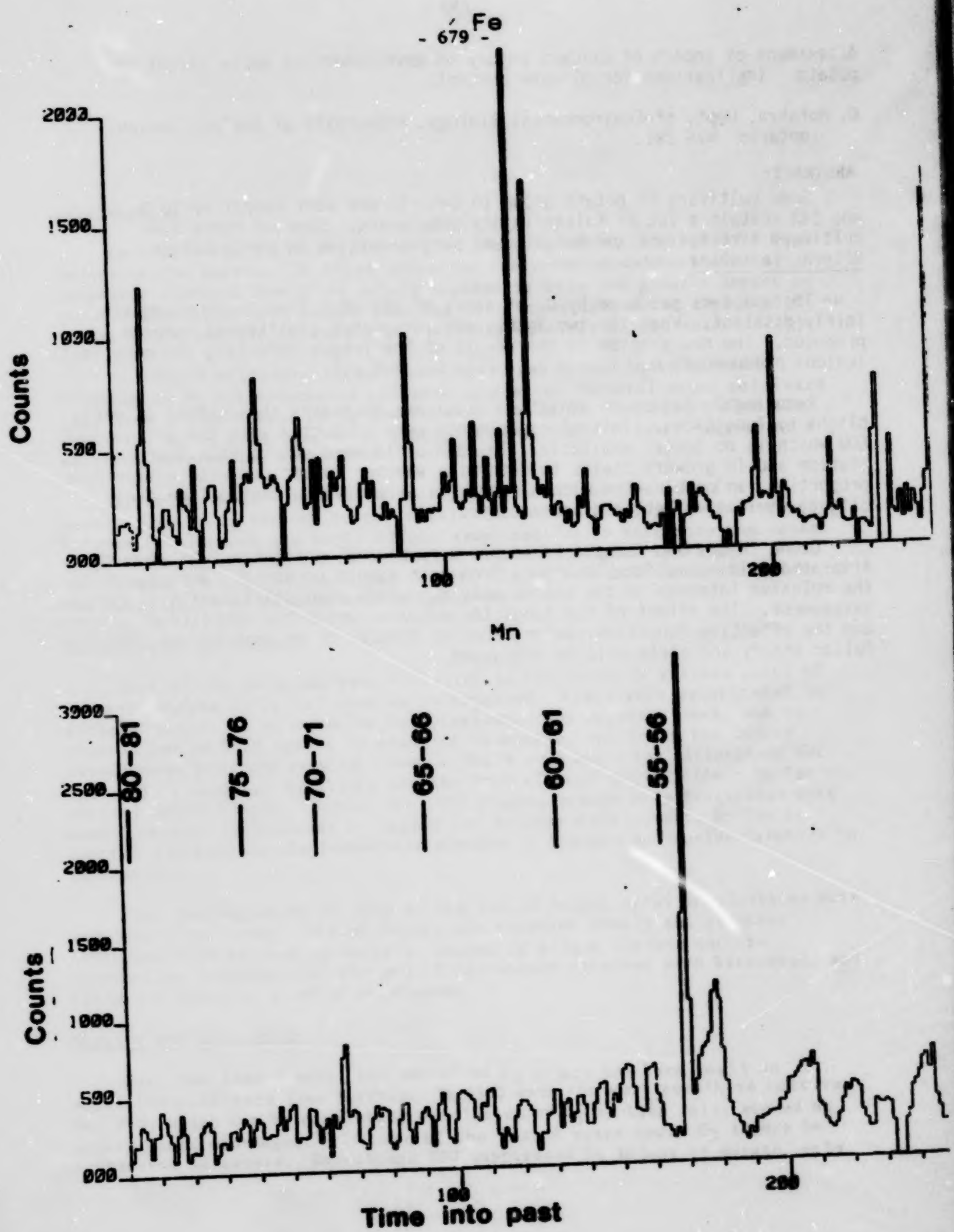

Fe
2000
1500
1000
500
000
Counts
100
200
Mn
3000
2500
2000
1500
1000
500
000
Counts
80-81
75-76
70-71
65-66
60-61
55-56
100
200
Time into past

FIGURE 7(b)

Assessment of impact of oxidant injury on development of early blight on potato: implications for disease control.

G. Hofstra, Dept. of Environmental Biology, University of Guelph, Guelph, ontario N1G 2W1.

ABSTRACT:

Some cultivars of potato grown in Ontario are very sensitive to O_3 and can sustain a lot of foliar injury some years. Some of these same cultivars like Norland and Norchip are very sensitive to early blight Alternaria solani.

The symptoms produced by O_3 or early blight when they occur alone are fairly distinct. When the two agents occur together a different symptom is produced. The new symptom is the result of the fungus infecting the many small lesions produced by O_3.

Reducing O_3 injury on sensitive cultivars increases the control of early blight by fungicides. Initial experiments were conducted with the antioxidant EDU which is no longer available. In 1983 plots were set up at a Research Station and in growers fields to determine whether fungicides with antioxidant properties, in combination with a fungicide effective in controlling early blight, would give better disease control.

Ozone injury and disease incidence were minimal until about mid-August. After that time considerable injury from both agents occurred. Because of the relative lateness in the season only two plots were still available for assessment. The effect of the fungicide manzate, which has antioxidant properties, and the effective fungicide, DuTer, applied singly or in combination, on foliar injury and yield will be discussed.

Introduction

In some crops yield suppression by ozone is directly related to O_3 injury on the leaves. In other crops the increased susceptibility to parasitic diseases due to O_3 injury appears to have the greater impact on yield. Over the last several years we have investigated the impact of O_3 on potato yield and the influence of O_3 injury on early blight epidemiology.

Sensitive cultivars like Norland have been deemed to be severely affected by O_3 but documented evidence is scarce. Several other cultivars also have a high degree of O_3 sensitivity (Table 1). Cultivars that are sensitive to O_3 also seem to be susceptible to early blight, suggesting a possible interaction between the two agents.

Experiments have been conducted (1) to determine the relative sensitivity to O_3 various potato cultivars in Ontario; (2) to distinguish between O_3 symptoms and early blight symptoms; (3) to determine the impact of O_3 in potato yield; (4) to establish whether an O_3/early blight interaction occurs and its significance; (5) to work out the implications of the interaction on disease control programs.

Experimental procedures

Most of the work has been conducted in the field in various parts of Ontario, backed up by lab studies as required. Plots were established in growers' fields or at experimental stations. The approach used, was to control one or both agents by means of chemicals, and to assess injury development over the growing season. While available the antioxidant EDU was used to suppress O_3 injury and the most effective fungicide - Du-Ter to control early blight. During the 1983 growing season only fungicides were used: Manzate to suppress O_3 injury and disease development; Du-Ter to control disease; or the formulated mixture of Manzate and Du-Ter (similar to Liromatin).

From the beginning of July to the end of August spray applications were made every two weeks. Foliar injury was assessed weekly and symptoms differentiated between O_3 stipple, expanding blight lesions and the interaction symptoms. At the end of the season potatoes were harvested, and yield and specific gravity determined.

Results and Discussion

Over the last 4 years the amount of O_3 injury has been small on sensitive cultivars like Norchip. In 1983 even the very sensitive cultivar Norland, which can be completely defoliated by O_3 by late July, showed no injury until mid-August. In none of the last 4 years could O_3 injury be considered as severe. Eventhough EDU suppressed O_3 injury on potato, only infrequently did it result in yield increases in the sensitive cultivar Norchip. The overall effect over 3 years was not significant (Table 2).

Reduction in O_3 injury by EDU frequently led to better control of early blight by the fungicide Du-Ter. Disease development was suppressed and yield and specific gravity enhanced (Table 3). Normally early blight shows up on potato leaves as a few large lesions which expand with time. When O_3 injury is first present many small blight lesions develop on the leaf, indicating that O_3 lesions become quickly colonized by the early blight pathogen.

That fact that an antioxidant in combination with a good fungicide gave better control of early blight, led to an assessment of available chemicals for more effective control. Manzate a chemical used for early blight control has been known for some time to have antioxidant properties. Du-Ter in our tests has been shown to be the most effective fungicide against early blight. A special formulation consisting of a mixture of these chemicals was made up for test purposes in 1983.

Initially 4 locations in Ontario were selected for testing: Grand Bend, Carlyle, Arkell and Alliston. Because of drought and early harvest by the grower, the plots at Grand Bend developed no O_3 injury and disease symptoms. The plots at Carlyle had insect and wilt problems and were also abandoned. The Alliston and Arkell plots were very dry most of the season resulting in low yields as in the commercially grown potatoes. O_3 injury became first apparent on August 17, but never consisted of more than 6% injury on Norland at Arkell and 30% at Alliston. Manzate significantly reduced O_3 injury (Table 4). With the longer nights and longer dew periods of late August early blight developed rapidly soon after O_3 injury was seen.

At the early stages of the disease Manzate appeared to give somewhat better control than Du-Ter, whereas once the disease progressed rapidly, Du-Ter and Liromatin gave better control (Table 4). It appears that if O_3 injury occurs before the early blight epidemic occurs a compound with antioxidant properties gives somewhat better control, presumably though a reduction in O_3 lesions. Once the disease is established the compound with the best fungicidal properties gives better control. The combination treatment (Liromatin) seemed to be the most effective in disease control, although often not statistically better than Du-Ter.

Yields were low with drought being the overriding faster. However, effects on yield of the treatments were still apparent in Norchip (Table 6). The different chemical treatments were not significantly different from each other for the 1983 season.

Summary

Drought, low levels of O_3 injury which occurred late, and rapid disease development in late August 1983 minimized differences between treatments.

Manzate reduced O_3 injury and appeared to give better disease control at the beginning of the epidemic.

The mix tended to give better disease control overall.

Reducing O_3 injury delays the onset of early blight epidemic. Therfore a fungicide with antioxidant properties is more effective early in the season. The fungicide most effective against the disease should be used once the epidemic has become establsihed.

Acknowledgement

The field work was conducted by J.D. Holley from 1980-1982 and J. Ford in 1983.

Publications arising from the project

Hofstra, G., R.T. Wukasch and D.M. Drexler. 1983. Ozone injury on potato foliage as influenced by the antioxidant EDU and sulphur dioxide. Can. J. Plant Pathol. 5:115-119.

Holley, J.D., R. Hall and G. Hofstra. 1983. Identification of rate-reducing resistance to early blight in potato. Can. J. Plant Pathol. 5:111-114.

Holley, J.D., G. Hofstra and R. Hall. Effects of ozone and early blight on foliar necrosis and tuber yield of potato. Phytopathol. In preparation.

Holley, J.D., R. Hall and G. Hofstra. Effect of leaf wetness and temperature on the rates of development of early blight on three potato cultivars. Can. J. Plant Pathol. In preparation.

Holley, J.D., G. Hofstra and R. Hall. The effect of oxidant injury on the appearance, fine structure, and development of early blight lesions on leaves of "Norchip" potato grown in the field. Can. J. Plant Pathol. In preparation.

Table 1: The relative O_3 sensitivity of various potato cultivars.

Cultivars	% Ozone injury Dates of Observation 6/8/81	 25/8/81
Norland	35.0	90.0
Campbell 13	21.0	50.0
Cherokee	25.0	40.0
Bel Rus	6.0	35.0
Monona	10.0	35.0
Norchip	9.0	30.0
Chieftain	8.0	25.0
Yukon Gold	12.0	20.0
Trent	7.0	20.0
Katadhin	9.0	12.0
Atlantic	6.0	8.0
Sebago	3.0	7.0
Superior	3.0	-
Rideau	1.0	7.0
Russet Burbank	0	3.0
Kennebec	0	2.0

Table 2: The effect of EDU, Du-Ter singly or in combination on yield in Norchip.

Location	Relative Row Weights			
	Check	EDU	Du-Ter	EDU + Du-Ter
Alliston 1980	19.50	20.90	22.00	23.90
Alliston 1981	14.00	12.50	16.00	17.50
Carlisle 1981	-	-	-	-
Alliston 1982	17.80	18.70	19.80	20.20
Burlington 1982	17.40	17.10	18.70	21.40
Means	17.20x	17.30x	19.10y	20.80y

LSD test at P = 0.05.

Table 3. The effect of EDU, Du-Ter singly or in combination on yield, specific gravity and infection rate on 3 cultivars (Norchip, Chieftain and Kennebec) over 3 years.

Treatment	Row Weight	Specific Gravity	Apparent Infection Rate
Check	20.38a	1.0686d	0.172g
EDU	19.97a	1.0689d	0.168g
Du-Ter	22.32b	1.0709e	0.148h
Du-Ter + EDU	22.63b	1.0716f	0.146h

LSD tests at P = 0.05

Table 4: Maximum observed ozone injury on Norland and Norchip potato at Arkell and Alliston, 1983.

	Norland	Norchip
Arkell	(August 22)	
Control	3.6 ab	2.1 a
Manzate	2.1 b	0.9 a
Du-Ter	6.3 a	1.0 a
Mix	4.0 ab	0.9 a
Alliston	(August 23)	
Control	31.9 a	5.5 a
Manzate	11.9 b	1.5 b
Du-Ter	20.0 ab	4.2 a
Mix	15.5 b	2.3 b

Differences significant at $P = 0.05$.

Table 5: Disease ratings partway through the early blight epidemic on Norland and Norchip potato at Arkell and Alliston, 1983.

	Norland	Norchip	
Arkell	(August 23)	(August 26)	(August 30)
Control	87.5 a	68.6 a	97.5 a
Manzate	39.0 b	17.4 b	80.0 b
Du-Ter	58.8 b	20.6 b	76.8 b
Mix	37.4 b	14.3 b	68.0 b
Alliston	(August 23)	(August 29)	(Sept. 5)
Control	87.5 a	11.3 a	66.9 a
Manzate	68.6 b	4.4 b	27.7 b
Du-Ter	71.2 b	4.4 b	15.8 c
Mix	64.2 b	4.6 b	13.5 c

Differences significant at P = 0.05

Table 6. Yield of Norland and Norchip potato at Arkell and Alliston.

	Norland	Norchip
Arkell	kg/row	
Control	10.2 a	7.8 b
Manzate	10.8 a	9.9 a
Du-Ter	9.9 a	9.7 a
Mix	10.6 a	9.3 ab
Alliston		
Control	9.5 a	11.0 b
Manzate	10.5 a	12.8 ab
Du-Ter	10.1 a	13.3 a
Mix	10.5 a	13.3 a

Differences significant at P = 0.05.

Production of Ozone Insensitive Field Bean Varieties

A presentation submitted to the Technology Transfer Conference No. 4,
Ontario Ministry of the Environment

T.E. MICHAELS, W.D. BEVERSDORF, B.D. MCKERSIE

Common bean (Phaseolus vulgaris L.) suffers foliar injury following episodes of high atmospheric ozone concentration. Bronzing, the reddish-brown stippling of the upper leaf surface is a symptom of the ozone injury. Bronzing is associated with loss of photosynthetic area, leaf abscission, and premature plant senescence.

White beans, also called navy or pea beans, are a common bean grown on 25,000 to 40,000 ha in southwestern Ontario. These beans are sold on Canadian and foreign markets for canning, and represent cash receipts of $30 million to Ontario farmers. Bronzing in white beans has been noted at ozone levels in excess of 9 pphm. The plants appear most susceptible during pod fill in mid to late August. Using the anti-oxidant ethylene diurea, G. Hofstra and others from the Department of Environmental Biology at the University of Guelph have estimated a 32% to 36% reduction in white bean yields as the result of ambient ozone levels at Elora and Ridgetown.

Though field beans in general are sensitive to ozone, insensitive cultivars have been found. These cultivars are of improper size, shape, color, or environmental adaptation for direct use in Ontario white bean production. However, this genetic variation in response to ozone can be exploited to develop ozone insensitive white bean cultivars adapted to Ontario by intercrossing insensitive with adapted, sensitive cultivars.

Experiments conducted by the Department of Crop Science at the University of Guelph with funding from the Ontario Ministry of the Environment were designed to identify genetic sources of ozone insensitivity, characterize the inheritance of insensitivity, initiate breeding populations incorporating insensitive sources and evaluate the cultivars derived from these populations.

Controlled environment studies and field trials were conducted with 162 field bean cultivars and plant introduction lines in 1978 and 1979. Snap bean cultivars were found generally less sensitive to ozone than white bean cultivars. The wax bean Gold Crop showed outstanding insensitivity to ozone. French Horticultural, Calima and Narda also exhibited little sensitivity. All white bean cultivars currently grown in Ontario were sensitive. The insensitive lines were intercrossed with the adapted white bean cultivars Seafarer, Ex Rico 23, and Kentwood to form breeding populations for inheritance studies and cultivar selection. Inheritance studies suggested that ozone insensitivity is controlled by multiple nuclear genes. Heritability estimates from controlled environment studies were high ($H = 0.66$ to 0.88) while estimates from field trials were lower ($H = 0.16$ to 0.21). These heritabilities suggest that selection for ozone insensitivity should be carried out in later generations of self pollination when individual plants are nearing homozygosity, and parental performance adequately predicts performance of progeny. We have chosen to integrate selection for ozone insensitivity with selection in the field for quality, architectural and yield characteristics, since these attributes must also be evaluated in plants nearing homozygosity.

Successful selection in the field relies upon cooperative environmental conditions, in this case, high ambient ozone levels. Conducting selection trials in multiple locations and years improves the probability of success. In 1983, over 1000 plots at Elora, Woodstock, Ailsa Craig, Mitchell and Palmerston were evaluated for insensitivity. Foliar injury was most apparent at the Woodstock breeding nursery and Mitchell yield trial. Two advanced line trials involving potential cultivars derived from Ex Rico 23 and Kentwood crosses with Narda were evaluated at Woodstock. Preliminary analyses indicate that most Ex Rico 23 x Narda derivatives were significantly less sensitive than Ex Rico 23. Kentwood was less sensitive than a Kentwood x Narda derivative (Table 1). These lines were selected for white bean quality, yield and disease resistance with little or no selection pressure against sensitivity. We would expect some lines not to surpass the parent for ozone insensitivity.

Integrated selection for quality, yield and ozone insensitivity was conducted this summer on 6 F_5 populations at Woodstock. The most insensitive lines from populations 79-1 (Narda x Eagle) and 79-2 (Narda x Provider) tended to show snap bean characteristics, but one population, 79-4 (Seafarer x Calima), contained two highly insensitive white bean type plants. Progeny of these plants will be evaluated and increased in 1984 for replicated quality, yield and insensitivity trials in 1985. Forty-five new F_5 populations will be evaluated for insensitivity in 1984, eight of which incorporate previously identified insensitive sources as parents.

Promising new sources of insensitivity were identified in the 1983 CIAT International Bean Flowering and Adaptation Nursery. The IBFAN was designed to assess the effect of latitude and climate on flowering, and included a wide range of germplasm. Five entries with early flowering dates and ozone insensitivity will be incorporated into the breeding program.

These initial efforts to improve ozone insensitivity of white beans constitute the first cycle of selection. First cycle selections can be inferior in quality or yield attributes if unfavorable germplasm is incorporated along with that conferring insensitivity. Second cycle selections, resulting from intercrossing lines selected in the first cycle, should show superior combinations of quality, yield and insensitivity.

Table 1. Ozone sensitivity of advanced breeding lines, Woodstock, Ontario, 1983.

Line	Background[1]	Rating[2]
W15-01	A	1.75
W07-01	B	2.00
W11-18	A	2.00
W13-03	A	2.25
W13-18	A	2.50
W07-03	B	2.50
W13-07	A	2.75
W15-19	A	3.00
W11-03	A	3.00
W11-02	A	3.25
W13-04	A	3.50
W07-08	C	3.75
W11-09	A	4.00
Seafarer		3.50
Kentwood		1.75
Ex Rico 23		3.75
$LSD_{0.05}$		0.75

1. A = Ex Rico 23 x Narda
   B = Ex Rico 23 x W77-08
   C = Kentwood x Narda

2. 0 = no bronzing, 5 = severe bronzing

Sweet Corn and Green and Wax Bean Responses to Air Pollution in Southern Ontario

D.P. Ormrod
Dept. of Horticultural Science
University of Guelph

Abstract

Replicated field experiments were conducted in 1982 and 1983 at the University of Guelph-Ontario Ministry of Agriculture and Food Research Station at Cambridge, Ontario; at the Ontario Ministry of Agriculture and Food Horticultural Experiment Station at Simcoe; and at the Canada Department of Agriculture Research Station at Harrow. Randomized block split-plot experiments were conducted with three concentrations of benomyl as a chemical protectant spray or drench, applied to plants weekly. Appropriate benomyl concentrations were established in laboratory studies with sweet corn and from literature references for beans. There were six cultivars of sweet corn and of snap beans at each location. Tobacco ozone-indicator plants were placed in each plot. The 1982 harvest data indicated that while there were substantial differences among yields of cultivars at each location, the relative differences were generally similar at all locations. The few changes in cultivar yield patterns may have reflected differences in temperature, rainfall, and other environmental responses, as well as differences in sentivity to air pollution. There were few statistically significant effects of benomyl sprays in these studies. The effect of 4000 ppm sprays was injurious in some cases, particularly in the sweet corn experiment at

Simcoe. Yield increases with 1000 ppm benomyl sprays were noted in some corn cultivars at Cambridge, and generally in corn at Harrow and in beans at Cambridge. Additional measurements made at the sweet corn harvest indicated that the benomyl effect on husk weight was always similar to that on total yield while cob number was significantly increased by benomyl only at Cambridge and cob size increased only at Harrow. Data analyses and interpretation for the 1983 field experiments are not yet complete, and will be reported later.

Introduction

Southern Ontario crop production areas are subjected to recurring ozone (O_3) episodes during the growing season. While the responses of many crops to O_3 in the field in southern Ontario have had considerable study in the past, those of sweet corn and green and wax beans have had little attention. The commercial production of sweet corn and green beans for the fresh market or for freezing or canning is an important horticultural activity in Ontario. In addition, both species are widely grown in home gardens. Studies elsewhere have indicated that at least some cultivars of each species are sensitive to O_3. Experiments in the field in southern Ontario are necessary to permit evaluation of a range of cultivars of each species under the weather and pollution conditions characteristic of this area. An understanding of cultivar sensitivities, location effects, weather interactions, amelioration techniques and various other air quality relationships is essential for an understanding of pollution limitations on productivity of these crops.

A study was initiated in 1982 to evaluate sweet corn and green and wax bean responses to O_3 pollution in southern Ontario by means of detailed observations of the nature and extent of injury during the 1982 and 1983 growing seasons, and by undertaking chemical protectant treatment experiments in the field. The objectives of the study were to determine yield and quality effects, to correlate observed injury with O_3 monitoring data taken at several locations, to evaluate cultivar sensitivity, and to determine the nature of leaf injury and growth retardation.

These objectives were approached by conducting field experiments in 1982 and 1983 at the Cambridge, Harrow and Simcoe Research Stations with evaluation of the response of six cultivars of each species to weekly applications of an O_3 protectant, benomyl. Data on yields and other response variables allowed some assessment of species, cultivar, chemical protectant, and location interactions.

Commercial Importance of Sweet Corn and Green and Wax Beans

Sweet corn is the third most important vegetable crop after tomatoes and potatoes, each of which has about three times the farm value of sweet corn. Approximately 17,000 hectares are grown with a farm value of about $18 million per year. Imports of sweet corn are limited to about 4% of the amount produced within the province. Sweet corn is well-adapted to southern Ontario and is an important contributor to farm income, as well as to the prosperity of the processing industry.

The green and wax bean crop is relatively less important than corn in Ontario accounting for only $5 million cash value to farmers. It could be of greater importance if imports could be replaced by local production. About one third of the green and wax beans consumed in Ontario are imported. Green and wax beans are considered to be well adapted to southern Ontario, yet some factors of the environment may be adversely affecting productivity and profitability. Sensitivity to recurring pollution episodes may be one such limiting factor.

Field Experiments

Replicated field experiments were conducted at the University of Guelph - Ontario Ministry of Agriculture and Food Research Station at Cambridge, Ontario; at the Ontario Ministry of Agriculture and Food Horticultural Experimental Station at Simcoe; and at the Canada Department of Agriculture Research Station at Harrow. At each location, randomized complete block split-plot experiments were conducted with weekly chemical protectant treatments, six cultivars of sweet corn with one planting date, six cultivars of green and wax beans with two planting dates in 1982 and one planting date in 1983, and six blocks in each experiment. The protectant treatments were sprays of water, 1000 ppm benomyl (a commercial fungicide that acts as an antiozonant), and 4000 ppm benomyl on both species in 1982, and sprays of 500 or 2000 ppm benomyl on beans but soil drenches of these concentrations on sweet corn in 1983. The sprays and drenches were applied weekly to all foliage on each plot. The cultivars were selected from the recommendation lists of the crop committees of the Ontario Ministry of Agriculture and Food.

The experimental design was a split-plot with the six cultivars as whole units and sprays as sub-units. There were three 3-meter long rows in each cultivar whole unit in 1982. In 1983 there were six rows in each whole unit with alternate rows untreated. Spray or drench treatments were sub-units with a different spray treatment on each row in 1982 or different spray or drench treatments on alternate rows in 1983. Row spacing was 90 cm for both species with 4 cm spacing between bean plants and 20 cm spacing between sweet corn plants. Tobacco indicator plants, cultivar 'Bel-W3', were placed on the perimeter of each plot.

Results

Sweet Corn

There was a highly significant cultivar effect at Cambridge with very large differences in yield among cultivars. 'Earlivee' was clearly least productive while 'Golden Jubilee', 'Beacon' and 'Flavorvee' were highest yielding. There was a significant cultivar x benomyl interaction because the total yields of some cultivars were very responsive to benomyl while others were not. The yields of 'Stylepak', 'Golden Jubilee' and 'Earlivee' were enhanced by benomyl. Husk weight, cob number and cob quality (length of cob covered with mature kernels) were highly significantly different among cultivars. Only husk weight and total yield were affected by the interaction. Cob number was significantly affected by benomyl, ~~and~~ while quality differed only among cultivars.

At Harrow, 'Earlivee' was also least productive but 'Beacon' was relatively much lower yielding than at Cambridge. In this case there was an overall significant effect of benomyl with the highest yield at 1000 ppm benomyl and lowest at 4000 ppm. In addition husk weight and quality but not cob number were significantly affected by benomyl.

At Simcoe there were highly significant effects of both cultivar and benomyl, with 'Golden Jubilee' and 'Flavorvee' the most productive. The overall benomyl effect was due to a reduction in yield with 4000 ppm benomyl. Of the three additional response variables, only husk weight was affected by benomyl application.

Green and Wax Beans

There were two plantings of beans, primarily because herbicide injury interfered with plant growth after the first planting at two of the locations. A second planting also allowed assessment of a wider range of O_3 episodes than would have been possible with a single planting, as beans have a shorter seeding to harvest cycle than sweet corn. The benomyl did not significantly affect yield in the first planting at Cambridge while there were highly significant differences among cultivars. 'Contender', 'Bush Blue Lake' and 'Tendergreen' yielded much better than 'Greencrop' or 'Cherokee Wax' while 'Kinghorn Wax' was intermediate. The first planting at Harrow had no significant benomyl effect but the best cultivar was 'Greencrop' with 'Kinghorn Wax' the lowest yielder. The post-planting pre-emergence herbicide was washed deeply into the soil by heavy rains at Simcoe and severely injured seedlings, especially those of 'Greencrop', 'Bush Blue Lake' and 'Cherokee Wax', with all yields reduced. There was not a significant benomyl effect.

In the second planting at Cambridge most cultivars yielded similarly with only 'Kinghorn Wax' and 'Greencrop' markedly lower yielding. In this case benomyl had a significant overall effect on yield with highest yield with 1000 ppm sprays and lowest with 4000 ppm sprays. At Harrow the array of cultivar differences was somewhat different from Cambridge but 'Kinghorn Wax' was lowest yielding at both locations. There was not a significant benomyl effect. In contrast, the second bean planting at Simcoe had no significant cultivar effect but there was a benomyl effect. The significant benomyl effect seemed to be largely due to an overall decreased yield with 4000 ppm benomyl sprays.

Injury on Ozone-Sensitive Tobacco Plants

Indicator plants of 'Bel W3' tobacco were injured similarly at all three locations. By mid-August all tobacco plants were severely injured up to leaf 10 with injury extending to leaf number 34 to 38. The extensive injury on the plants indicated that substantial doses of O_3 had been received at all locations.

Ozone Concentrations in Southern Ontario in 1982 and 1983

Monitoring data and hourly plots for four Ontario stations were obtained from the Ontario Ministry of the Environment-Air Resources Branch. The Simcoe data were intended to relate to the field experiments at Simcoe. The Kitchener data were for the experiments at the Cambridge Research Station and the Windsor and Merlin data for the experiments at the Harrow Research Station. The hourly plots revealed that there were a number of potentially injurious O_3 episodes in both years. In 1982 the number of hours with an O_3 concentration greater than 80 ppb was highest at Simcoe followed by Windsor but the ppb-hr dose was about the same at the two locations. The other two locations had much lower durations and doses in 1982. The principal episodes at Simcoe in 1982 occurred in mid-June and mid-July while at Windsor the episodes were in mid-June and early July. The only substantial episode at Kitchener in 1982 was in mid-June.

Ozone concentrations in 1983 were higher in June but not much different in July, August and September, compared with 1982. The plants were in the seedling stage during the June episodes and visible injury symptoms were noted. However, preliminary examination of yield data indicates that the early O_3 injury was not reflected in final yield differences between sprayed and unsprayed plants.

Evaluation of Contaminated Water
and
Soil Sites as Sources
of
Airborne Hazardous Materials

D. Mackay
A. Hughes
J. Phyper

Department of Chemical Engineering and Applied Chemistry
University of Toronto

ABSTRACT

The status of an experimental program supported by the Ontario Ministry of the Environment Air Resources Branch is described in which the aims are to develop methods of quantifying emissions (i.e. source strengths) of organic contaminants from water bodies and from soils. The approach has been to design and operate a small scale system in which water containing dissolved contaminant is subjected to air and water turbulence which can be adjusted to simulate any desired environmental condition, as may exist in a lake, pond, river or lagoon. A similar system has been devised for soils. Attempts are being made to develop relatively simple mathematical models which can be used to correlate the experimental data and predict emission rates in other systems from a knowledge of the water or soil properties and the contaminant's physical chemical properties.

INTRODUCTION.

Although most airborne contaminant sources are "point" in nature, there may be significant emissions to the atmosphere from "non-point" sources such as lakes, rivers or soil. These "non-point" sources may be low in terms of "grams per square metre per second" but since the area is potentially large and the duration long, it is possible that the emissions may represent substantial fractions of the total industrial, municipal, domestic or agricultural emission.

Our aim is to devise two test procedures or protocols which can be used by an environmental agency to predict the likely emission rates by a combination of experiment and calculation based on a sound theoretical basis. The project is planned for three years and at this point in time it is approximately half complete. The water test is nearly complete while the soil test is less advanced.

WATER TEST.

Mass Transfer Coefficient Correlation.

The first stage was a review of the existing literature which was facilitated by a recent EPA sponsored project which we had just completed (Mackay et al. 1982). We also reviewed available test procedures and highlighted some deficiencies in them in a paper co-authored with Smith of SRI (Smith et al. 1983). An important aspect has been the gathering and critical review of data correlating mass transfer coefficients (MTCS) for air-water exchange. A paper describing this aspect was written up and has been published this year in Environmental Science and Technology (Mackay and Yeun 1983). In this work we demonstrated a fundamental difference between laboratory and environmental

MTCs as shown in Figure 1 which is reproduced from that paper. Data have also been gathered for wind speeds over land and water in Ontario in order that these speeds can be inserted into the correlation equations to obtain an estimate of prevailing MTCs under any given wind speed and fetch conditions.

It should be noted that it is accepted that it is necessary to define two mass transfer coefficients; for the air phase (K_G) and the water phase (K_L) and that the reciprocals of these coefficients (i.e. resistances) add in series (with a correction for relative concentrations in the two phases in the form of the Henry's Law Constant (H) or air-water partition coefficient (K_{AW} or H/RT). This represents the Whitman "Two Resistance Approach". In all cases an overall or total resistance or conductivity or MTC is obtained experimentally which may consist of varying proportions of water and air resistance.

EXPERIMENTAL SYSTEMS.

A system was desired to study volatilization rates of organic contaminants that exhibit a wide range of air-water partition coefficients, i.e., Henry's Constant (H). In general, substances with large values of H volatilize rapidly at a rate controlled by the water phase MTC (K_L) and those with low values of H volatilize slowly at a rate controlled by K_G. In this way, the effects of liquid phase and gas phase diffusion control can be examined and applied to the Two Resistance Theory for interphase mass transfer. It is thus desirable to measure the volatilization rates of a series of compounds of varying H in order that from the measured overall MTC the individual values of K_G and K_L can be extracted.

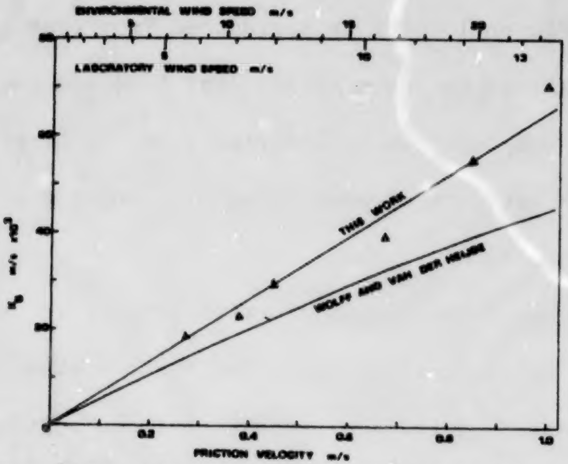


Figure 1a Present K_G correlation (Schmidt number 0.6) and that of Wolff and van der Heijde

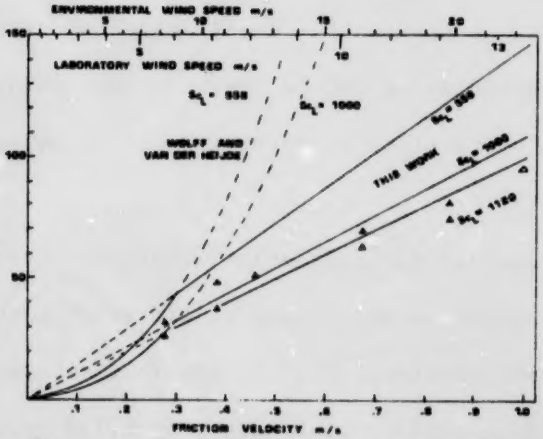


Figure 1b Present K_L correlation and that of Wolff and van der Heijde

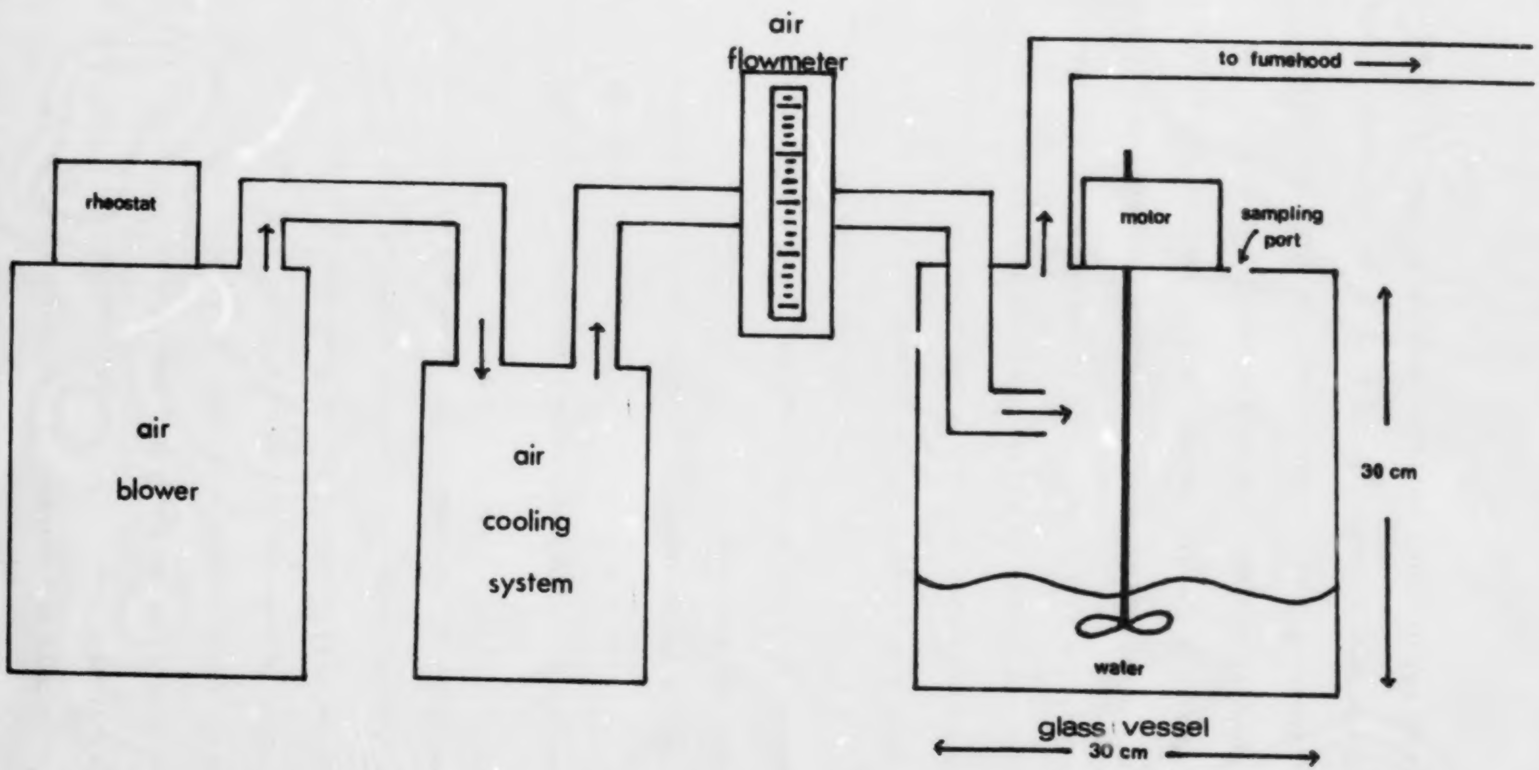
Reprinted from **ENVIRONMENTAL SCIENCE & TECHNOLOGY**, Vol. *17*, Page 211, April, 1983

An air-water volatilization rate apparatus (Figure 1) has been built and refined. The system is designed to examine the process of volatilization under a variety of conditions. Air phase turbulence can be varied through the use of a blower connected to a rheostat and a flowmeter (calibrated for volumetric air flow rates). Water turbulence can be varied through the use of a propeller powered by a motor and connected to a revolution counter. The system can easily be adapted to study the effects of temperature on the volatilization process as both the air flow and aqueous reservoir can be immersed in temperature-controlled water baths.

Two different analytical methods, gas chromatography (G.C.) and high pressure liquid chromatography (H.P.L.C.) have been used for quantitative analysis. A Hewlett Packard 5840A gas chromatograph and integrator, with a purge and trap sampler Model 7675A is used for the detection of the highly volatile substances. The gas chromatograph is equipped with a flame ionization detector and contains a SE-30 capillary column, with nitrogen as the carrier gas. During the past year, much work has been devoted to perfecting the G.C. systems for the detection of chemicals from the water samples from the volatilization apparatus. The HPLC system is used for the analysis of lower volatility substances such as polyaromatic hydrocarbons and alcohols. The HPLC system consists of an Eldex dual high pressure pump in conjugation with a LDC UV detector (254 nm) and a Hewlett Packard 3390A Integrator. The mobile phase is methyl alcohol: water (80 : 20) and the column is C18 μ Bondapak.

In a typical experiment we measure the decrease in concentration of a mixture of chemicals, present in true solution form, in the aqueous phase.

FIGURE 1


rheostat
air
blower
air
cooling
system
air
flowmeter
to fumehood
motor
sampling
port
30 cm
water
glass vessel
30 cm

No attempt is made to analyse the air phase. Six litres of distilled water along with an aqueous solution saturated with the substances of interest are added to the glass vessel. Blower speeds and stirrer speeds are set and remain constant during the entire experimental run. During the run, water samples are taken from the glass vessel with a gas-tight syringe and analyzed by either the G.C. or the HPLC system. The concentration decrease with time for each chemical is monitored and a volatilization rate or "overall air-water mass transfer coefficient" is calculated from the slope of the semi-logarithmic plot of concentration versus time.

The time needed for a given experimental run depends on whether rapidly or slowly volatilizing substances are being studied, and ranges from a few hours to several days. In order to explore the effects of liquid phase and gas phase diffusional resistance it is necessary to study a given group of substances under a NxN matrix of air turbulence (i.e blower speeds) and water turbulence (i.e. stirring rates) conditions.

The solutes used have been benzene, toluene, methylcyclopentane, naphthalene, phenanthrene and n pentanol. Unfortunately, there is no convenient hydrocarbon solute (to our knowledge) which has a Henry's Law Constant somewhat smaller than that of phenonthrene. Such a solute would be invaluable in assisting the accurate extraction of K_G values.

RESULTS.

The results have been compiled in the form of overall MTCs from which mean K_G and K_L values have been extracted using the planned variation in H. The K_G and K_L values have been correlated against air rate (A) and stirrer speed (S) using two equations of the form

$$K_L \text{ or } K_G = K_1 + K_2A^nS^m$$

where there is a total of 8 constants (i.e. pairs of K_1,K_2,n and m).

A contour plot of K_L versus K_G can then be obtained as shown in Figure 3. On this figure is also plotted the normal range of Ontario lake conditions, as obtained from meteorological data and the K_G and K_L correlations. It is thus possible to adjust conditions in the test system to simulate any desired condition of MTCs and temperature. It is also possible to use natural water (rather than distilled water) to simulate the actual emission conditions.

DISCUSSION.

Present work consists of reprocessing the data to include a diffusivity or Schmidt Number correction factor in the analysis, the investigation of temperature effects, the use of various natural waters and an exploratory study of the effects of surface films. A final test protocol will be written early in 1984. It is expected that the solute of interest will be tested along with three other "bench mark" solutes, probably toluene, naphthalene and phenanthrene. It will then be possible to undertake an experimental investigation of solute emissions from the actual water of concern at the desired temperature and turbulence conditions and obtain experimental data which can be subjected to rigorous theoretical interpretation.

SOIL TEST.

The development of a soil test protocol is more difficult because of the heterogeneous nature of the emitting medium. Two experimental approaches have been taken. The first was the use of a test system

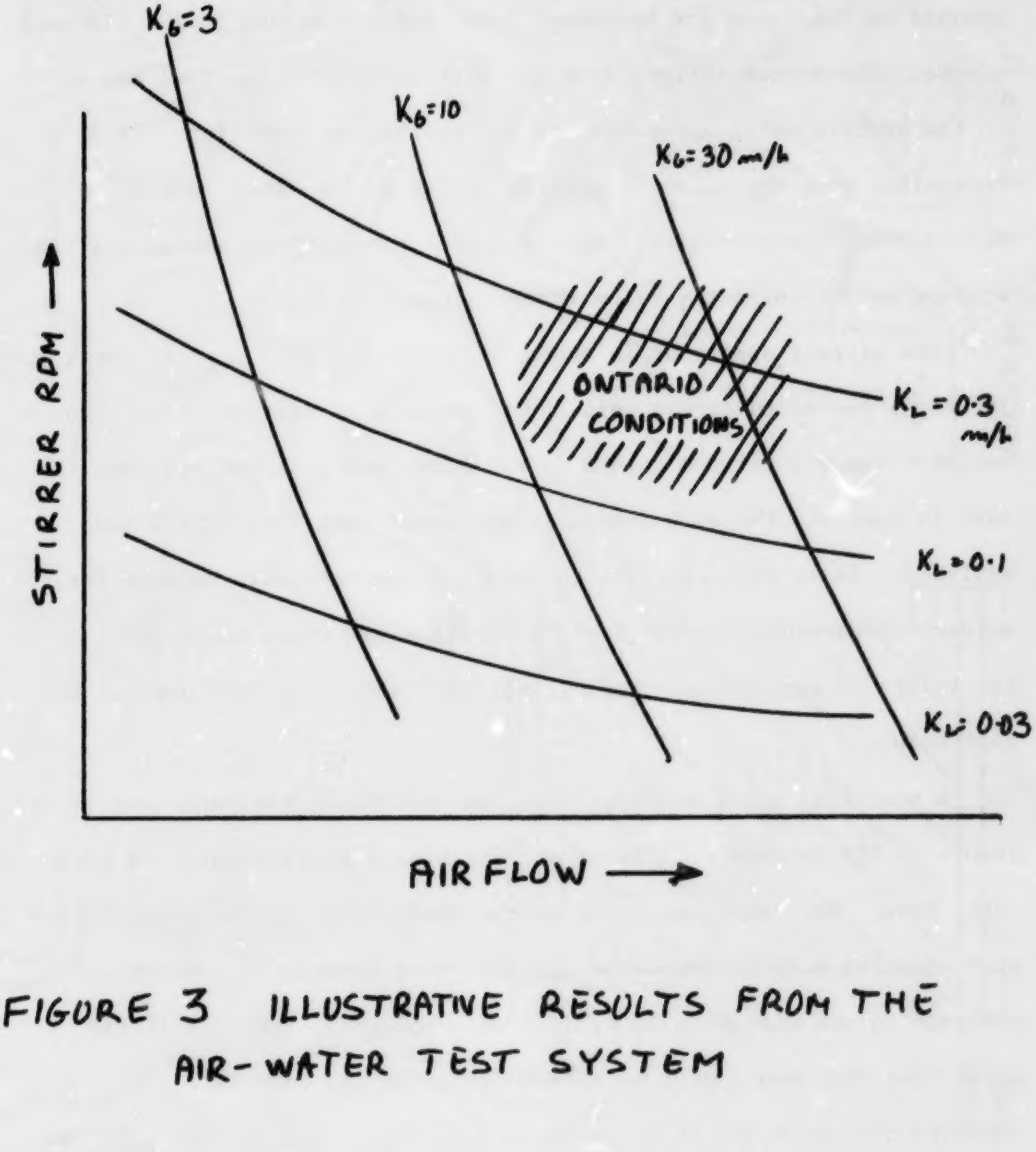


FIGURE 3 ILLUSTRATIVE RESULTS FROM THE AIR-WATER TEST SYSTEM

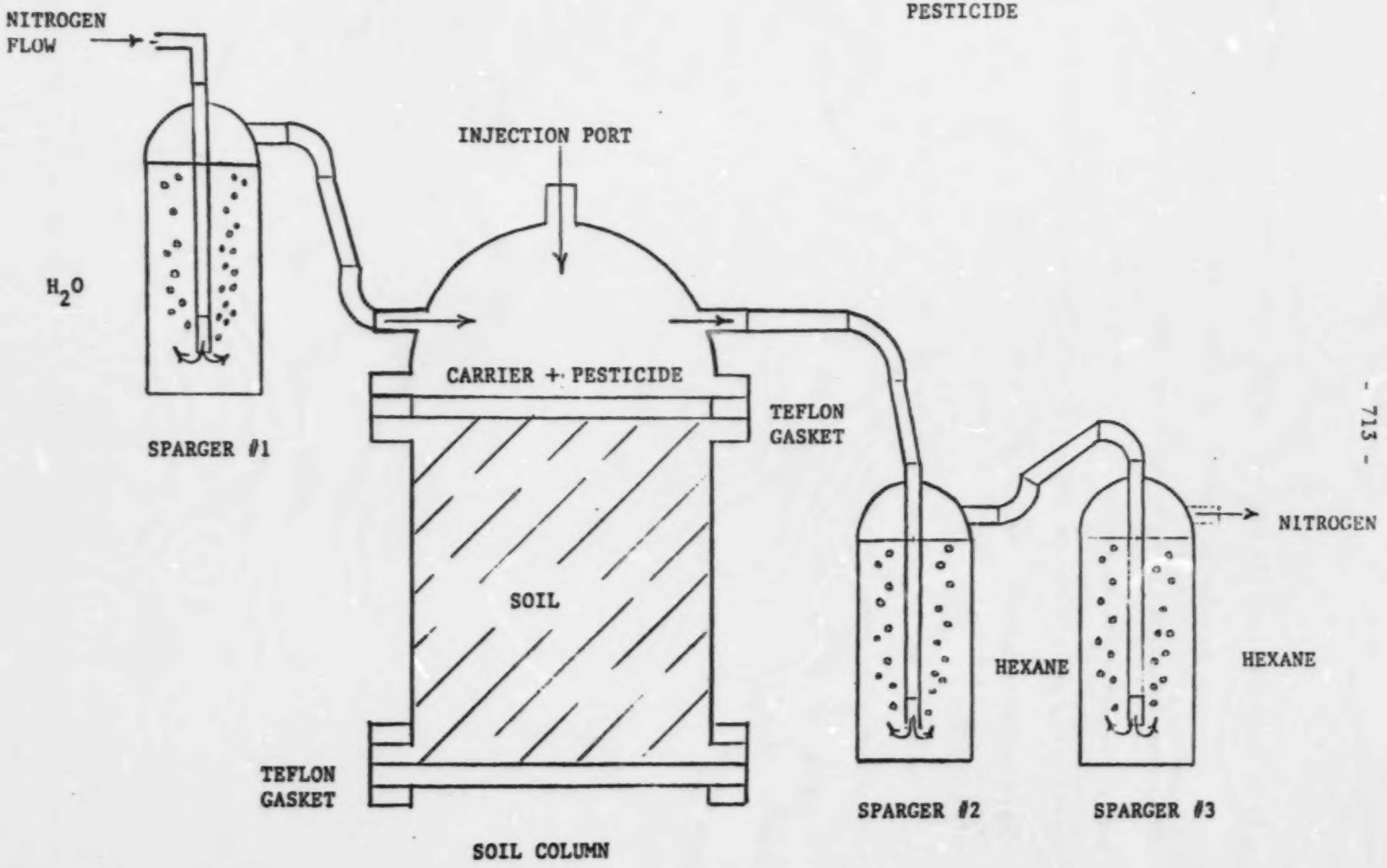
similar to that used for the water test, but containing soil. Although simple, this system suffers from the difficulty that the soil may not be in the desired water saturation or sub-saturation condition. It is recognized that the water "condition" plays an important role in determining volatilization. Accordingly a more complex system has been devised and is currently being investigated.

The present apparatus is shown in Figure 4. In a typical test, the solute(s) are added to the soil "neat" or in a carrier solution such as hexane. The volatilized solute is collected and analysed and when the test is complete the soil column is sectioned into 5 mm layers and analysed. It is thus possible to obtain a complete mass balance for the solute. Separately the equilibrium sorption partition coefficient of the solute is determined. Radio-labelled lindane has been used as the test solute.

A numerical model has been compiled describing the behaviour of the solute in the surface soil layer and includes migration into the saturated zone. This model is based on the traditional differential diffusion equation with allowance for partitioning between air, water and soil, and solute migration in air and water phases. There is little doubt that this model will be able to describe and correlate the observed phenomena but it is probably too complex for general use. We envisage that it may be simplified by rewriting the equations in a fugacity form, then further simplified to obtain equations which are more suitable for routine rather than research use.

It is envisaged that the final test protocol will be similar in principle to that of the water test, but that the experimental procedure

FIGURE 4
APPARATUS FOR
SOIL-AIR EXCHANGE OF
PESTICIDE


NITROGEN FLOW
H_2O
SPARGER #1
INJECTION PORT
CARRIER + PESTICIDE
TEFLON GASKET
SOIL
TEFLON GASKET
SOIL COLUMN
NITROGEN
HEXANE
HEXANE
SPARGER #2
SPARGER #3

will be more complex. It may consist of taking a sample of the desired soil, adjusting its "water condition", adding the solute of interest with other "bench mark" solutes, determining the diffusion, volatilization characteristics, separately determining the air-water-sorption partitioning properties and finally reconciling the experimental data with a relatively simple mathematical model.

CONCLUSIONS.

The water system is nearing completion with a full description of the recommended procedure expected in early 1984. Most effort is now being devoted to the soil test system in expectation of completion in early 1985.

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Smith, J. H., Mackay, D., Ng, C. W. K. "Volatilization of Pesticides from Water" Residue Reviews 85 73 (1982)

Mackay, D., Yeun, A. T. K. "Mass Transfer Coefficient Correlations for Volatilization of Organic Solutes from Water" Environ. Sci. Technol 17 211 (1983)

EVALUATION OF ALTERNATIVES TO HIVOL SAMPLING FOR PAH

C.S. Davis, R.B. Caton, S.G. Guerin
Concord Scientific Corporation
2 Tippett Road
Downsview, Ontario
M3H 2V2

and

W.C. Tam
Ontario Ministry of the Environment
Air Resources Branch
880 Bay Street
Toronto, Ontario

INTRODUCTION

One of the most commonly used methods for sampling airborne polynuclear aromatic hydrocarbons (PAH) is the Hi-Vol sampler. This method is fraught with deficiencies because of vapour-phase losses of the more volatile PAH compounds and the reaction of collected material with gases such as ozone, nitrogen dioxide and even nitric acid (van Vaeck et al, 1980; Cautreels and van Cauwenberghe, 1978; Lee et al 1980; Peters and Seifert, 1980; Barton et al, 1978; Brorstrom et al, 1983; Van Vaeck et al, 1979). Most attempts at rectifying these deficiencies have relied on solid sorbents such as Tenax (Cautreels and Van Cauwenberghe, 1978), polyurethane foam (PUF) (Krstulovic et al, 1977; Lindgren et al, 1980; Thrane and Mikalsen, 1981; Yamasaki et al 1982) and XAD-2 Pellizzari E.D., 1979) to capture the volatilized PAH compounds. This approach is, however, still susceptible to the reaction of ozone and other reactive gases with the collected material both on the filter and on the solid sorbent (Jager and Harris 1980; Hughes et al, 1980).

In an effort to develop instrumentation that would mitigate these problems, a research program was undertaken by Concord Scientific Corporation through funding provided by the Ontario Ministry of the Environment, to modify the standard Hi-Vol sampler. These modifications would provide an improved sampling method for PAH while utilizing currently available instrumentation and also allow the collection of large enough samples for chemical and other analyses, such as bioassays.

The modifications to the standard Hi-Vol sampler involved the addition of a backup solid sorbent cartridge and an ozone denuder. The solid sorbents investigated were PUF, XAD-2 and Florisil. Initialy, the use of C_{18} GC Bondapak on Porasil was considered, but its high cost and the reported high pressure drop across this material (Lindgren et al) rendered it unsuitable for routine use in a network of modified Hi-Vol samplers.

The provision of an ozone denuder preceeding the Hi-Vol filter required the complete design, development and fabrication of this device. The selective removal of ozone from ambient air during sampling has not been reported previously. It was therefore necessary to find a suitable material that would act as a near perfect sink for ozone yet not remove any vapour phase PAH from the sample air stream.

Laboratory and field tests are described in which candidate solid sorbents (PUF, XAD-2 and Florisil) were investigated and laboratory and field ozone denuders were developed and tested. Field evaluation of the modified Hi-Vol samplers is also described.

EXPERIMENTAL

Construction of samplers: Laboratory Scale Sampler

Laboratory scale samplers consisted of a glass fibre filter (47 mm diameter) contained in a polyethylene filter holder and a series of 1 to 3 custom fabricated stainless steel cartridges. The cartridges were fabricated to allow separate amounts of the sorbents to be used. See Figure 1. The sample flow rate through the laboratory scale samplers was such that the face velocity was similar to that in a Hi-Vol sampler operating at a nominal 20 CFM flowrate.

Construction of modified Hi-Vol samplers

The standard Hi-Vol sampler was modified by placing a cartridge between the filter support (plenum) and the motor. Figure 2 shows the cartridge system used. This system allows great flexibility in the amounts of sorbent that may be employed and was accomplished by the use of variable lengths of a polyethylene insert and as many screens (to separate each sorbent bed) as desired.

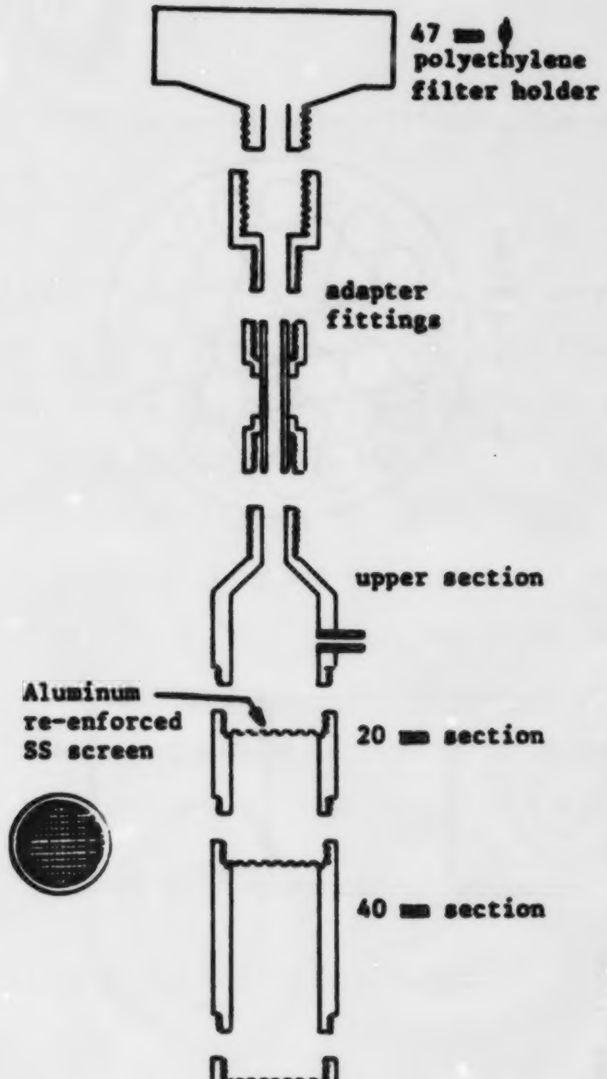
47 mm ϕ
polyethylene
filter holder
adapter
fittings
upper section
Aluminum
re-enforced
SS screen
20 mm section
40 mm section
50 mm section
Lower section

Figure 1 Schematic of Laboratory Test Chamber Filter/Sorbent Cartridge

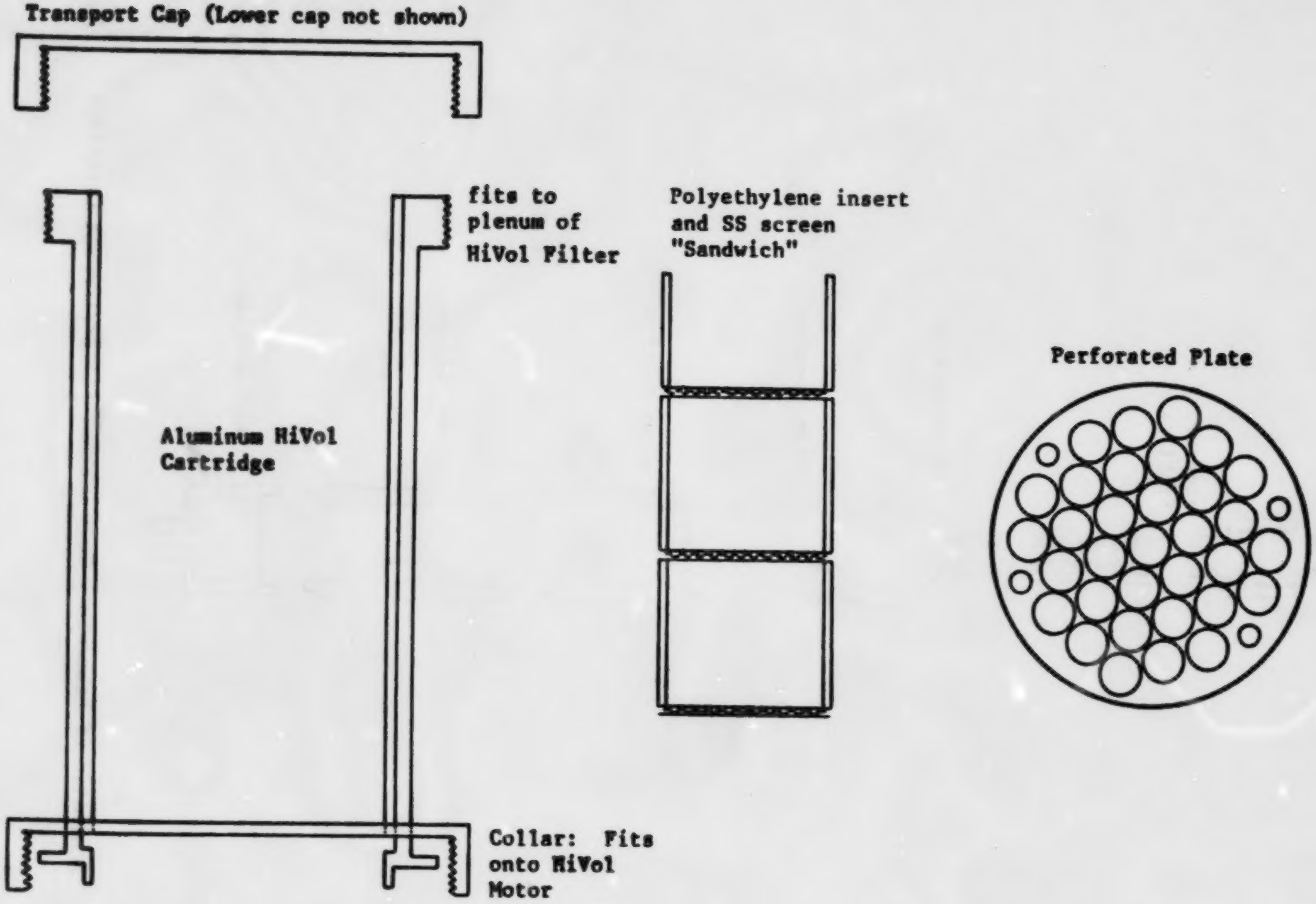
Transport Cap (Lower cap not shown)
fits to plenum of HiVol Filter
Polyethylene insert and SS screen "Sandwich"
Aluminum HiVol Cartridge
Perforated Plate
Collar: Fits onto HiVol Motor

Figure 2 Details of HiVol Sorbent Cartridge

The field scale denuder-Hi-Vol-cartridge system developed is illustrated in Figure 3.

The standard Hi-Vol housing was extended and covered with the standard Hi-Vol cover to protect the denuder bundle and filter from the elements. The denuder bundle consisted of over 1250 Kraft paper tubes, each 61 cm long and 7.9 mm ID. The intersticial channels in the bundle were sealed so that all the air flow was through the tubes. The denuder bundle was seated in a support tray and the seating was rendered airtight to ensure that all the airflow through the filter and cartridge was directed through the denuder tubes. Measurements of the air velocity 1 cm above the bundle were made while sampling at 15 cfm. Measurements taken at 16 locations were in a 5% range, indicating even flow through the complete bundle.

Field Sampling and Laboratory Experiments

Field sampling was conducted on the roof of the Hamilton Beach Rescue Unit Association (HBRUA) building on Beach Boulevard in Hamilton. Sites in Hamilton have been shown to have significant ambient PAH concentrations as a result of nearby coke oven emissions (Katz et al. 1980). The details of the location of the sampling site relative to the MOE ambient monitoring station and the Stelco and Dofasco facilities are shown in Figure 4.

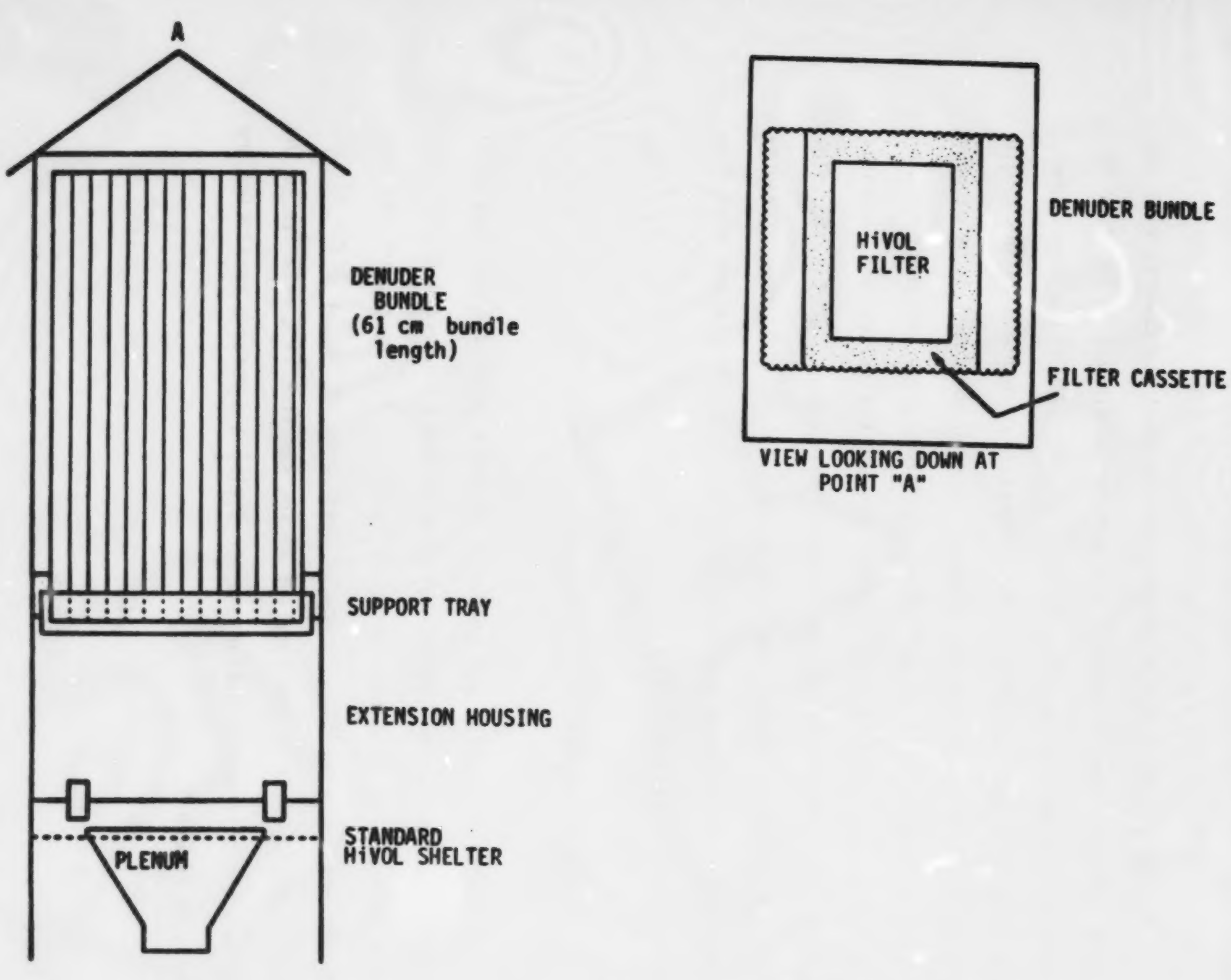
A
DENUDER BUNDLE (61 cm bundle length)
SUPPORT TRAY
EXTENSION HOUSING
STANDARD HiVOL SHELTER
PLENUM
TO HiVOL MOTOR
HiVOL FILTER
DENUDER BUNDLE
FILTER CASSETTE
VIEW LOOKING DOWN AT POINT "A"

Figure 3 SCHEMATIC OF HiVOL OZONE DEUNDER

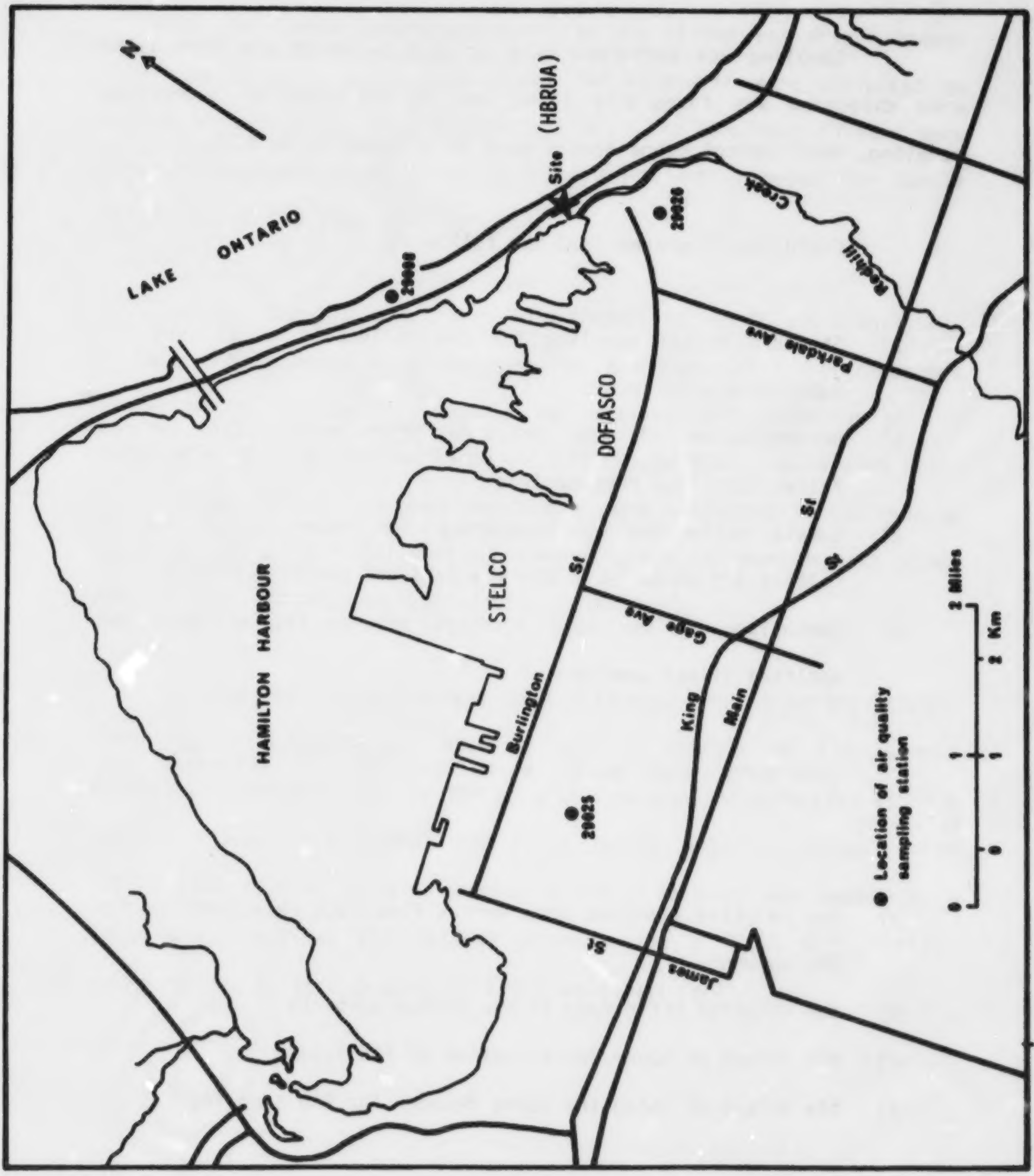
N
LAKE ONTARIO
Site (HBRUA)
29000
29026
Redhill Creek
Parkdale Ave
DOFASCO
STELCO
HAMILTON HARBOUR
Burlington St
Gage Ave
King St
Main St
James St
29025
● Location of air quality sampling station
0 1 2 Km
0 1 2 Miles

Figure 4

Location of Sampling Site in Relation to Existing MOE Sites and Major Pollution Sources

Sampling was performed only on days on which the forecasted wind direction was favourable (SSW) and in the case of summertime sampling, when ambient ozone levels were anticipated to be high.

Field sampling comprised the following:

a) Standard Hi-Vol sampling for the collection of filters for laboratory experiments
b) Determination of the pressure drop across the various filter/cartridge combinations
c) Sample collection for comparing the retention of PAH on filters and sorbents in the standard and modified Hi-Vols
d) Comparison of the mass loadings between the standard and modified Hi-Vol samplers

Laboratory experiments were conducted to determine the following:

a) the relative pressure drop versus flow rate relationships for the sorbents
b) the relative efficiency of the backup sorbents
c) the effect of ozone concentration on PAH losses
d) the effect of using the ozone denuder for PAH sampling

For these laboratory runs an air stream was drawn through PAH-laden filters. The volatilized PAH compounds were collected on backup sorbent plugs or a cryogenic trap. The PAH-laden filters were cut (as required) from Hi-Vol filters which were exposed for sample collection at the site near Hamilton.

All samples were stored in a freezer at -15 °C until analysed. Filters and sorbents were analysed for a minimum of 6 PAH compounds (selected to include carcinogenic or mutagenic PAH compounds with a range of ring structure and hence volatility) by HPLC coupled with fluorescence detection. Samples and blanks were extracted in cyclohexane (soxhlet extraction) followed by concentration to near dryness under reduced pressure and resolubilization in methanol.

The HPLC chromatograms were obtained using a Waters ALC/GPC 244C liquid chromatograph equipped with a 254/375 nm fluorescence detector. Excitation was at 254 nm with fluorescence detection using a 375 nm filter. A Radial Pak 5 µm column with a methanol/water (75:25 v/v) eluting solvent grading to 100 % methanol was used. At a later stage a Vydac C18 201TP54 column using a 50:50 acetonitrile: water eluting solvent grading to 100 % water was used.

RESULTS AND DISCUSSION

The relationships between the pressure drop across a 2 cm bed depth of sorbent and the flow rate were examined for the three sorbents-PUF, XAD-2 and Florisil using the laboratory scale sampler. (See Figure 5). As expected, PUF has the lowest pressure drop and florisil the highest. These tests indicated what practical amounts of sorbents could be used in Hi-Vol samplers. It should be recognized that the life of Hi-Vol motors is highly dependent on the pressure drop across the glass fibre filter/sorbent cartridge combination.

Each of the modified Hi-Vols contained two or three sections of the same sorbent. For these modified Hi-Vol samplers, the typical relationships between the measured pressure drop (indicated as the vacuum in inches of water) for various sorbents and the flow rate are indicated in Table 1. A flow rate of 15-20 cfm was the target and this was easily achieved with ~ 2.5 cm bed depths of XAD-2 and PUF or 2 cm of florisil.

The relative performance of the three sorbents was evaluated given the requirement for a sampling rate of 15 - 20 cfm. Three separate sections of PUF and XAD-2 and two sections for florisil backup sorbents were employed in field runs. Analytical data are presented in Table 2 in which the flow rates and the percentages of each of six PAH compounds retained by the filter and each sorbent section are tabulated.

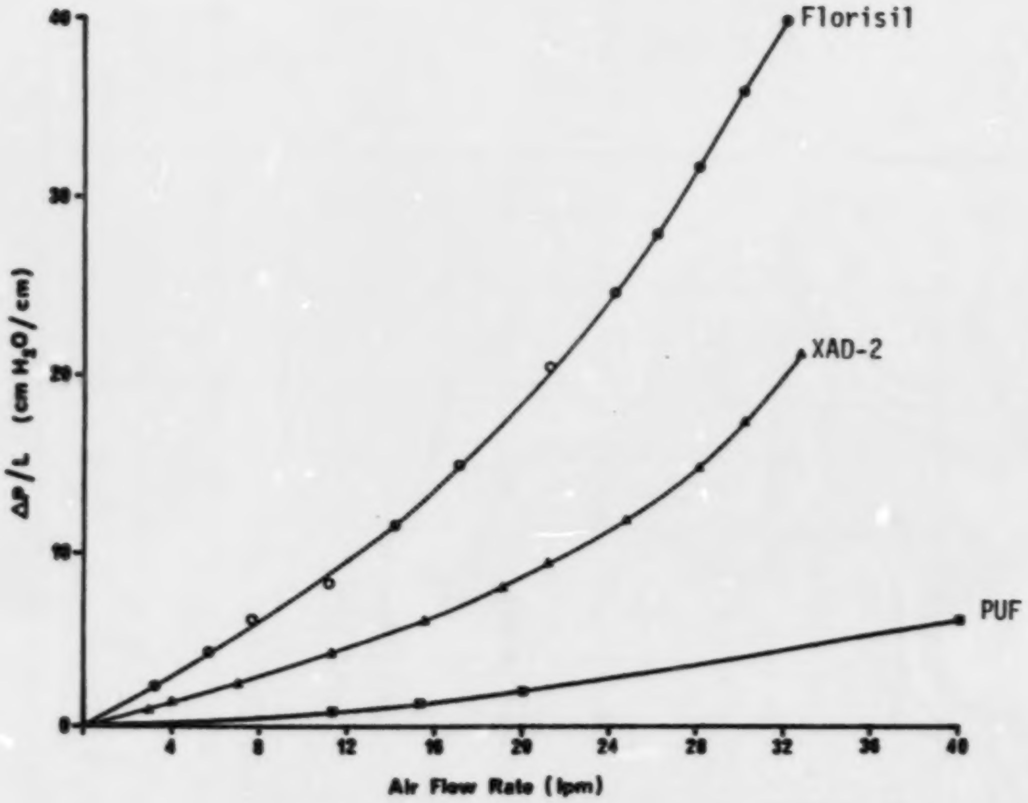

Figure 5   Relationship Between Pressure Drop and Flow Rate for Sorbents

Table 1

Pressure Drop in Various Hi-Vol Filter/Sorbent Cartridge Samplers

Test Item	Measured Vacuum " H_2O	Measured Flow Rate (CFM)
Filter, empty cartridge	20	63-65
Filter, cartridge with		
2.54 cm depth foam (4.06 g)	43	35-37
5.08 cm depth (8.1 g)	>50	21-23
11.2 cm depth (16.2 g)	>50	13-15
Filter, cartridge with 2.54 cm depth XAD 2 (39.4 g)	>50	24-26
Filter, cartridge with 2.54 cm depth florisil (58.6 g)	>50	10-12
Filter, cartridge with 2.54 cm depth foam (4.06 g)	20	20**
Filter, cartridge with 44 g XAD (~2.8 cm bed)	43.5	20**
Filter, cartridge with 38 g florisil (~1.6 cm bed)	40	20**

** Flow set to 20 CFM.

Table 2

Relative Performance of Various Filter/Sorbent Cartridge Systems

Sampler	S #	Sample	% of Total Analysed					
			F	P	B(a)P	3 MC	B(ghi)P	1 2 4 5-D
Std. H-Vol	41	Filter	100	100	100	100	100	100
			(4.5)*	(4.6)	(3.2)	(0.8)	(1.6)	(2.7)
PUF	8	Filter	21	11	91	87	< 64	86
		Section 1	78	87	4	< 6	< 25	8
		Section 2	0.5	0.3	< 0.3	< 2	< 6	3
		Section 3	1	1	5	5	< 6	3
			(59)	(80)	(8.1)	(1.0)	(5.8)	(5.1)
XAD-2	14.5	Filter	11	11	54	55	54	64
		Section 1	82	81	35	29	40	28
		Section 2	5	5	7	8	3	4
		Section 3	2	4	4	9	3	4
			(35)	(26)	(4.6)	(0.6)	(5.9)	(1.9)
Florisil	16	Filter	23	30	99.1	94	77	92
		Section 1	62	41	< 1	< 5	21	7
		Section 2	15	29	0.1	< 0.6	2	1
			(35)	(24)	(5.6)	(0.8)	(3.2)	(3.5)

S# = Sampling rate in cfm
F = Fluoranthene
P = Pyrene
B(a)P = Benzo(a)pyrene
3 MC = 3-Methylcholanthrene
B(ghi)P = Benzo(ghi)perylene
1245D = 1,2,4,5 - Dibenzopyrene
* Numbers in parentheses are total concentration in ng/m^3

The data in Table 2 indicate that most losses from the filter are incurred by fluoranthene (F) and pyrene (P). PUF appears to be most effective in capturing PAH compounds but this may be due to a lower sampling rate for the particular test. For the less volatile PAH compounds, the standard Hi-Vol yielded PAH concentrations similar to those obtained using the filter/sorbent cartridge system.

A modified Hi-Vol (with 2 sections of XAD-2 back up sorbent) and an Andersen cascade impactor (Hi-Vol with Andersen head) were compared in side-by-side sampling. The PAH collected on the filters are given in Table 3. The filter in modified Hi-Vol collected about 50% more of each PAH than the Andersen filters. All filters in the Anderson sampler were combined for the analysis. It should be noted however that the sampling rate for the Andersen sampler is nearly three times larger than that for the modified Hi-Vol. Analogous comparative experiments by Katz and Chan (Katz & Chan, 1980) indicated that the Andersen sampler collected nearly twice as much PAH than the standard Hi-Vol. However in those experiments, the Andersen sampler was operated at the lower flowrate (by a factor of 2.5). It is likely that the sampling rate will be dominant in determining the losses of PAH from filters.

The effects of ozone on the retention of PAH compounds on the filter and sorbents and the efficacy of the use of the ozone denuder were studied under laboratory and field conditions. The PAH losses from PAH-laden filters through which air with or without ozone was passed are given in Table 4. For this laboratory study, all the PAH-laden filter discs used (47 mm diameter) were obtained from a single Hi-Vol filter. The second column in Table 4 gives the mean and standard deviation of the weight of each PAH on three other discs (47 mm diameter) cut from the same Hi-Vol filter as that used for the loss experiments.

The data in the third and fifth columns, (labelled U) in Table 4 are the weights of PAH on discs with the same history (except for passage of air through them) as the discs used in columns labelled E_1, E_{2A} and E_{2B}.

In the absence of ozone (columns U and E_1), there were the expected losses of flouranthene and pyrene similar to those indicated in Table 2. These losses were recovered on backup florisil sorbent cartridge (first section). For the heavier PAH compounds, there were no apparent losses from the filters (back up sorbent cartridges showed no detectable quantities of these PAH compounds).

Table 3

Collection of Selected PAH on Filters from Modified HiVol and Andersen Cascade Impactor

PAH	Concentration (ng/m^3)		Ratio
	HVX*	HVA#	HVX/HVA
F	13.6	8.5	1.6
P	11.3	7.5	1.5
B(a)P	5.9	4.7	1.3
3MC	1.9	1.3	1.5
1245-D	2.6	2.0	1.3
B(b)F	11.6	8.0	1.5
B(k)F	6.4	4.4	1.5

* HiVol with XAD-2 cartridge. 570 m^3 sample volume. Flow rate 14 cfm

# HiVol with Anderson Head. 1620 m^3 sample volume. Flow rate 40 cfm

F = Fluorene
P = Pyrene
B(a)P = Benzo(a)pyrene
3MC = 3 Methylcholanthrene
1245D = 1,2,4,5 Dibenzopyrene
B(b)F = Benzo(b)fluoranthene
B(k)F = Benzo(k)fluoranthene

Table 4

Effects of Ozone on Retention of PAH Compounds in Laboratory Filter/Sorbent Cartridge Sampling Systems

PAH	Weight in ng on Filter					
	Mean ± SD	U	E_1	U	E_{2A}	E_{2B}
F	162 ± 34	142	50	108	54	58
P	126 ± 31	54	32	87	15	52
B(a)P	103 ± 25	112	123	119	48	47
3 MC	48 ± 10	25	30	27	15	14
B(ghi)P	<36 ± 14	9	6	ND	ND	ND
1 2 4 5-D	65 ± 17	85	97	65	32	33

U	Unexposed filter
E_1	Exposed filter, $[O_3] = 0$
E_{2A}	Exposed filter, $[O_3] = 190$ ppb
E_{2B}	Exposed filter, $[O_3] = 190$ ppb
ND	Not Detected

F = Fluoranthene

B(a)P = Benzo(a)pyrene

B(ghi)P = Benzo(ghi)perylene

P = Pyrene

3 MC = 3-Methylcholanthrene

1245D = 1,2,4,5 - Dibenzopyrene

For the runs in which ozone was present (columns E_{2A}, E_{2B}), there were losses of the heavier PAH compounds from the filter and none of these compounds were detected in the backup sorbents. In the case of fluoranthene and pyrene, the losses are similar to those in the absence of ozone but the back-up sorbent (florisil) collected substantially more fluoranthene (especially) and pyrene than was expected to be lost from the filter. It is suspected that an interfering compound in the HPLC analysis may account for this observation.

Ozone Denuder Material Evaluation

Several materials were evaluated as candidates for use in the ozone denuder. Screening tests were conducted using inlet ozone concentrations of up to 450 ppb at temperatures ranging from 0 to 30 °C. Two of the most promising materials - latex and Kraft tubing -were selected for more exhaustive and stringent testing. Table 5 summarizes the removal efficiencies obtained under varying conditions of temperature, duration of testing and $[O_3]$. Paper-based tubing was found to be superior, however kraft tubing was mechanically stronger and more readily available, and was therefore utilized on the construction of the full-scale denuder.

Table 5

Typical Results of Removal Efficiency of Candidate O_3 Denuder Materials

Tube Material	Temp. °C	Duration hrs	$[O_3]$ ppb	Removal Eff. %
Kraft	22	24	166	70
	-15°C	24	127	52
Paper	22	25	117	74
	-15	2	117	62
Latex	22	24	133	83
	-15	1	157	7
Latex-MnO_2	22	24	126	95 - 100
	-15	3	125	13

Results from a laboratory experiment in which a small scale ozone denuder was employed in front of the PAH laden filter discs at the same time that another disc was exposed to ozone, are given in Table 6. The columns U, E and D refer respectively to filter discs which were unexposed, exposed to 190 ppb O_3, and exposed with a denuder in place.

The data clearly show that the denuder is effective in reducing losses of PAH compounds.

Field runs in which the modified Hi-Vols with and without the ozone denuder in place were conducted to determine the effectiveness of the ozone denuder. These runs were performed in the late summer on days when ambient temperatures and ozone concentrations were relatively high. During the sampling period, the mean ozone concentrations at the nearest MOE monitoring station ranged from 20 to 55 ppb and the mean temperatures ranged from 22 to 29 °C. Analytical data from these experiments are not yet available.

Measurements of ozone concentrations at the inlet to the ozone denuder and in the space between the outlet of the denuder and the filter paper indicated a removal efficiency of ~ 80%. Ambient concentrations were ~ 30 ppb. Similar measurements in which the ambient air stream was spiked with ozone from an ozone generator, gave removal efficiencies of 63 - 71% at inlet ozone concentrations of 30 to 70 ppb. The

Table 6

Laboratory Experiments to Investigate Effectiveness of Ozone Denuder in Reducing PAH losses from Filters

PAH	Weight in ng on Filter				
	Mean ± SD	U	E	E	D
F	162 ± 34	99	17	22	41
P	126 ± 31	133	35	39	78
B(a)P	103 ± 25	108	14	18	54
3 MC	48 ± 10	33	9	12	27
B(ghi)P	36 ± 14	ND	ND	ND	ND
1245-D	65 ± 17	53	8	9	26

U Unexposed filter

E Exposed filter $[O_3]$ = 190 ppb

E Exposed filter $[O_3]$ = 190 ppb

D Exposed filter $[O_3]$ = 190 ppb but preceded by denuder

F = Fluoranthene

P = Pyrene

B(a)P = Benzo(a)pyrene

3 MC = 3-Methylcholanthrene

B(ghi)P = Benzo(ghi)perylene

1245D = 1,2,4,5 - Dibenzopyrene

maintenance of constant ozone concentration at the inlet of the ozone denuder was difficult under field conditions. Analagous laboratory experiements conducted in an 8 m^3 chamber in which stable ozone concentrations between 100 and 140 ppb were established, showed that the removal efficiency of the Hi-Vol ozone denuder was better than 98%.

SUMMARY AND CONCLUSIONS

The standard Hi-Vol sampler can easily be modified by the addition of a versatile sorbent cartridge and if required an ozone denuder. PUF was found to be the most appropriate sorbent (compared to XAD-2 and Florisil) in view of its greater efficiency in trapping volatilized PAH compounds and also due to its lower resistance to flow. The use of PUF will allow adequate flow rates (15-20) cfm) without significantly reducing the life of Hi-Vol motors.

Laboratory and field experiments with an ozone denuder utilizing kraft paper as the sink for ozone demonstrated the efficency of the denuder in reducing the ozone concentration to which the PAH compounds (on the filter and on the sorbent) are exposed. The addition of an ozone denuder and backup sorbent cartridge to a Hi-Vol sampler have been demonstrated to be easily accomplished. The costs for these modifications are not likely to be excessive.

These modifications should significantly improve the methodology for ambient PAH sampling. The extension of the application of the denuder technology to the removal of NO_2 from the air stream will represent the next major contribution to the advancement of the sampling methodology for PAH. These advancements are extremely important in view of the reported higher mutagencity of some nitro PAH compounds (Schuetzle, 1983). It is therefore esential to establish whether the nitro- as well as oxygenated PAH species do occur in the atmosphere or whether they are artifacts resulting from the reactions of NO_2 and O_3 with material collected on the filters.

ACKNOWLEDGEMENT

All HPLC analyses were carried out by Mr. Steve Jenkins of IEC-Beak Analytical Services, Toronto.

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Laser Induced Emission Spectroscopy of Polycyclic Aromatic Hydrocarbons (PAH) in Low Temperature Matrices

by F.J. Morgan, S.V. Filseth, and C.M. Sadowski
York University, Downsview, Ontario M3J 1P3

ABSTRACT:

Accurate identification and determination of trace amounts of PAH compounds in environmental samples is a prerequisite to any sensible attempt to set clean air standards. Emission and absorption spectroscopy have provided satisfactory data for inorganic compounds, but have been hampered from doing so for PAH compounds by the broad spectral features of such compounds at room temperature. Moreover, characteristic spectral lines are needed for a useful identification and determination technique. Shopolskii spectroscopy, in which samples are dissolved in normal albeanes (e.g. heptane) and then cooled to low temperatures (15^{o}K), can give the necessary narrow line-like features. Then, radiation of any wavelength shorter than the threshold for a transition from the ground state of the PAH compound to its first singlet electronic level can be used to produce emission characteristic of that compound. However, some excitation wavelengths will be more effective than others, and these wavelengths will vary from compound to compound. Hence a tunable, high intensity, narrow band excitation source is desirable. We use a pulsed, tunable dye laser in conjunction with a closed cycle helium refrigerator to investigate Shpolskii spectra of individual PAH compounds. Then, these spectra can be used to identify and determine particular PAH compounds present in environmental samples. We shall describe our technique, discuss our results for some PAH compounds, and also describe our initial attempts to analyse environmental samples obtained in the Hamilton, Ontario area by Concord Scientific Corportation of Toronto.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) occur as trace pollutants in the atmosphere, in soil, and in water.(1) Some of them are known carcinogens e.g. benzo(a)pyrene or mutagens [e.g. dibenz (a,h) anthracene]. Their derivatives and heterocyclic relatives [e.g. 1-nitrobenzo(a) pyrene] can be even more potent as mutagens, and possible carcinogens (2). To set sensible air quality standards, we need to know what levels of these PAH compounds can be tolerated, and what levels exist in the local environment. The former need is medical and biological in character, and is outside our expertise. It is the latter problem, largely analytical in nature, which has interested us.

PAH compounds arise from combustion process, and from atmospheric chemical reactions. The same is true of their derivatives and related heterocyclic compounds. They, along with derivative and related compounds, number in the thousands. This huge number presents considerable analytic difficulty in their identification and quantification. Chromatography in its various forms and in conjunction with fluorescence spectrometry and/or mass spectrometry has been a standard tool for analysis. Continual refinements to the basic chromatographic techniques have been required to keep abrest of ever increasing problems (2). Nonetheless, the resolution of chromatographic techniques is limited, and the number of PAH compounds is enormous. One, or even more than one, additional technique seems essential. Such a technique, or techniques, need not be considered an alternative but rather a complement in the difficult task of analysis for PAH compounds.

Shpol'skii spectroscopy (3,4) allied to a tunable dye laser excitation source appears to be one such additional technique. It offers the convenience of "line-like" spectra whose wavelength position can be used for identification and whose intensity can be used for quantification. We have been using such a system for several months to investigate its suitability for the analysis of atmospheric samples obtained from a Hi Vol sampler.

SHPOL'SKII SPECTROSCOPY

Atomic absorption or emission spectra are routinely used to analyse samples for such elements as lead, tin, nickel, etc. The sharp, isolated spectral features of such elements permit unambiguous identification of a given element, and the intensity of the features is a measure of the element's abundance. Granted, problems created by the 'interference' of other materials do arise, but these difficulties are not insuperable. Unfortunately, PAH compounds do not produce such "line-like" spectra in solutions at room temperature. These large molecules have a host of possible vibrational and rotational states, many of which overlap one another in wavelength (i.e. 'interfere'). Often such molecules are present as solutes in some organic solvent and this causes further 'interferences' due to the interactions between solvent and solute. Consequently room temperature spectra of solutions containing PAH compounds have broad features which make it impossible to identify which compounds are present in a complicated mixture obtained from ambient air (3,4). A different approach is needed for spectroscopic analysis.

The Shpol'skii effect provides a means for a new approach. PAH compounds, dissolved in a normal alkane at low concentration ($\leq 10^{-5}$M.), are cooled to temperatures of several degrees Kelvin before their spectra are obtained. This has two important effects. Firstly, the PAH compound is rigidly located in the frozen solid. Hence it does not rotate. Moreover, it is in its ground state at this low temperature, i.e. minimum electronic, vibrational, rotational energy. The lack of rotation leads to vibrational levels within a molecule which are free of rotational structure. Secondly, the similarity in structure between the PAH solute and the normal alkane solvent allows the PAH molecule to "fit into" the matrix of the frozen solvent (6). Thus there are a small number of orientations of the structural axis of the PAH molecule realtive to the matrix of the frozen solvent. In turn, this leads to an equally small number of interaction energies between the solute and solvent.

As a consequence of these two effects, the spectra obtained from individual PAH molecules have a "line-like" character. This 'line-like' character arises because the individual vibrational energy levels of the molecule have no rotational structure to 'blur' their separation. Equally important, there are a small number of orientations of the solute relative to the solvent. Each orientation produces a shift in the set of energy levels of a PAH molecule, and so each orientation leads to a shift in the wavelength positions of the fluorescence. Were there a large number of orientations, the correspondingly large number of shifted wavelengths would blur into one another and so produce rather broad spectral features. Instead, there will be a small number of shifts which will lead to intense, discrete spectral features. In turn, that gives the sort of spectra needed for analytical spectroscopy.

EXCITATION AND EMISSION SPECTRA

Spectra from isolated PAH molecules have features which can be understood in terms of general ideas governing their absorption and emission properties. Since those ideas led to our strategy for the use of Sphol'skii spectroscopy in studying PAH compounds, a synopsis of them seems appropriate.

A common model for the absorption and any subsequent radiation by a large molecule is based on Kasha's view (7) that radiation occurs from the lowest energy level in any electronic state of a large molecule contained in a liquid or solid. This arises from fast (picosecond time scale) collisional relaxation processess between the molecule and its surroundings that leave the molecule in its lowest level of an electronic state. The excess energy is dissipated to its surroundings.

An examination of figure 1 will clarify this. Since the PAH molecule sits in a frozen solid at 15°K (approximately), it absorbs radiation from the lowest vibrational level of its ground state (step 1). Clearly, the longest wavelength for this step will be $\lambda_o = \frac{hc}{E_{00}}$. Following this excitation process the molecule relaxes to the lowest level of the singlet electronic state S_1 (step 2). From this lowest level it might undergo a crossing to the overlapping levels of the triplet electronic state T_1 (step 3B) or the singlet electronic state S_o. In the former event, there follows a rapid, non-radiative relaxation to the lowest level of T_1 (step 3). In the latter event rapid relaxation to the ground state of S_o occurs. Whichever happens the molecule is now in the lowest level of the appropriate electronic state. If this state is above the ground state then a radiative transition can occur, either fluorescence (step 3A) or phosphorescence (step 4). A similar process follows an initial excitation into the second excited electronic state (S_2).

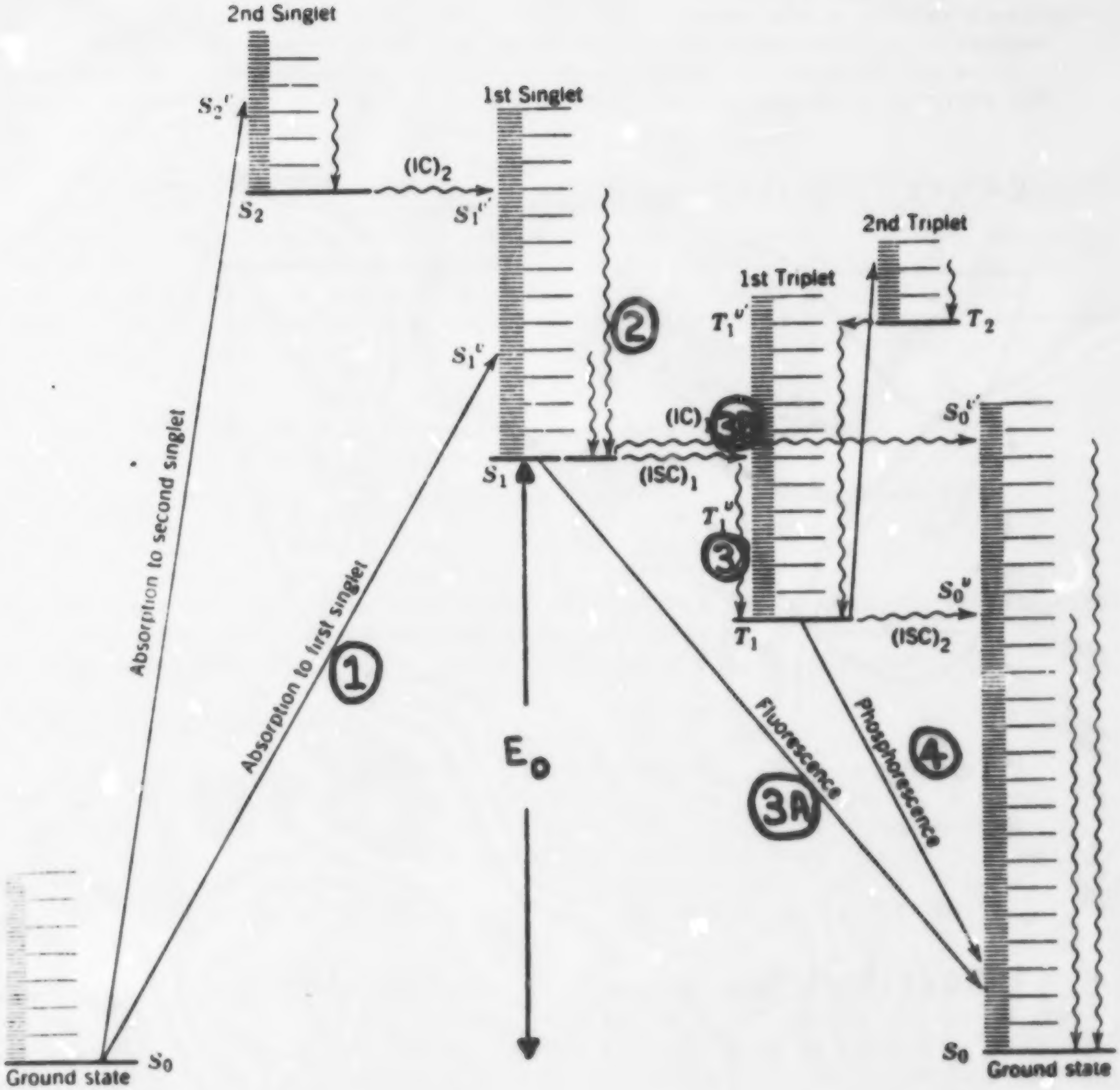
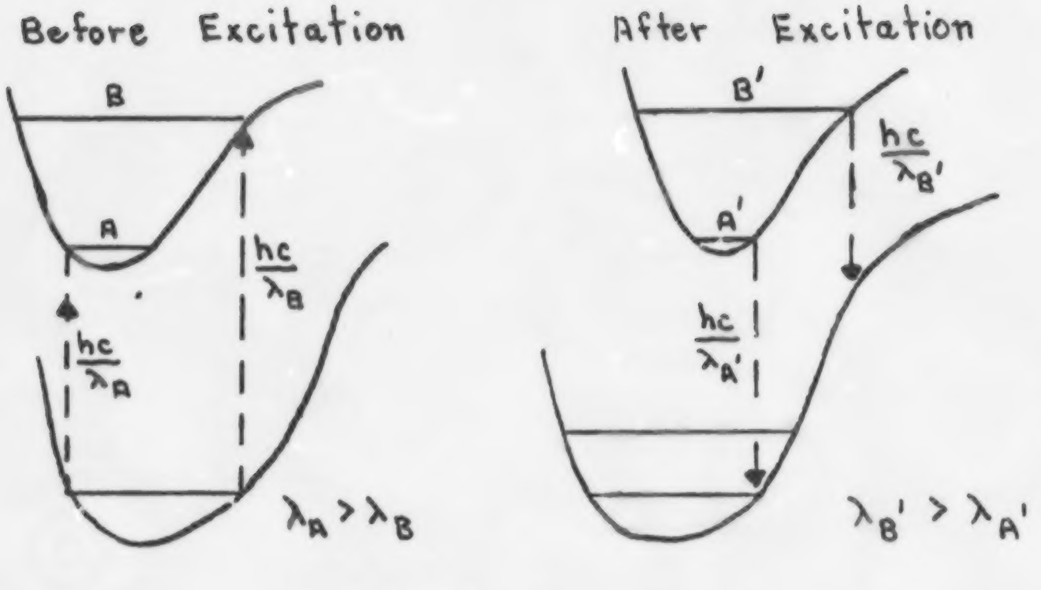

2nd Singlet
S2v'
1st Singlet
(IC)2
S2
S1v''
2nd Triplet
1st Triplet
T1v'
T2
②
S1v
(IC)
S0v'
S1
(ISC)1
T1v
③
S0v
T1
(ISC)2
Absorption to second singlet
Absorption to first singlet
①
E0
Fluorescence
Phosphorescence
④
3A
S0
Ground state
S0
Ground state

Figure 1 Energy Levels



Before Excitation

After Excitation

B
A
$\frac{hc}{\lambda_B}$
$\frac{hc}{\lambda_A}$
$\lambda_A > \lambda_B$
B'
A'
$\frac{hc}{\lambda_{B'}}$
$\frac{hc}{\lambda_{A'}}$
$\lambda_{B'} > \lambda_{A'}$

Figure 2 Potential Wells

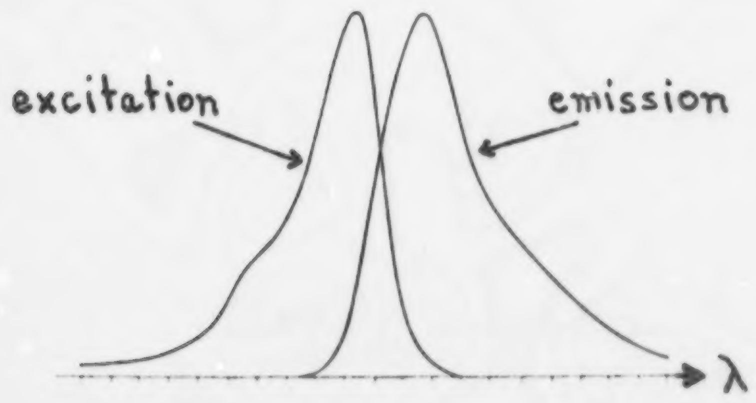


Figure 3 "Mirror Image" Spectra

As a consequence of radiative emission from the lowest excited singlet or triplet state all spectra for a given PAH molecule should have the same features regardless of the initial excitation wavelength (provided it is no larger than λ_o). However, some excitation wavelengths have a greater probability of absorption than others, and these excitation wavelengths will produce more intense fluorescence or phosphorescence. Hence a change in the wavelength of excitation will not change the spectral features in a spectrum but will change the intensity of those features.

Most large molecules undergo an increase in internuclear separation following excitation. The increased internuclear separation, along with the Franck - Condon principle for transitions between different rovibronic energy states, leads to a "mirror-image" relationship between absorption and emission spectra (see figure 2) from a given molecule (8). Figure 3 clarifies this point. The excitation wavelength λ_1 and λ_2 (absorption) become the fluorescence wavelengths λ_1' and λ_2' as a result of a changed internuclear separation in the excited state.

We used these ideas, particularly those coming from Kasha's rule, to develop the following strategy for our study.

1. Prepare laboratory solutions of pure, individual PAH compounds.
2. Irradiate a frozen sample.
3. Record the excitation spectrum.
4. Record the fluorescence spectrum.
5. Choose a combination of excitation and fluorescence wavelengths to selectively detect one PAH compound in the presence of others.
6. Apply the results of step 5 to an environmentally obtained sample.

EXPERIMENTAL PROCEDURE

Laboratory samples were prepared by weighing a few mg. of the PAH compound, (Aldrich Chemical or individually donated) dissolving it in normal Heptane (Caledon-193), and then diluting the stock solution to the desired concentration. Following this, a few ml. of the sample were placed in a small pyrex ampoule and de-gassed by alternate freezing to 77°K (liquid nitrogen) and melting at low pressure while pumping. The ampoule was sealed under vacuum.

Environmental samples were obtained from Concord Scientific (Toronto) who had used a Hi Vol sampler in the Hamilton, Ontario area in the late winter of 1983 to obtain them (9). These samples came to us after extraction and subsequent elution by liquid chromatography. We used our basic sample preparation procedure (see foregoing paragraph), but incorporated three different solvent techniques. Firstly, we tried methanol as the solvent since the environmental samples had come to us in methanol. Secondly, we tried dilution of the methanol sample in normal heptane. Thirdly, we evaporated the sample to dryness and redissolved it in normal heptane.

A closed cycle helium refrigerator (CTI, model 21SC) was used to cool the sample contained in the pyrex ampoule. The ampoule was placed in a small copper block which was attached to the cold head of the refrigerator, the whole system being inside a vacuum chamber. Operating temperatures of 15°K were typical, the temperature being measured with a calibrated silicon diode (Lakeshore Cryotronics, model DT 500). In practise the sample was plunged

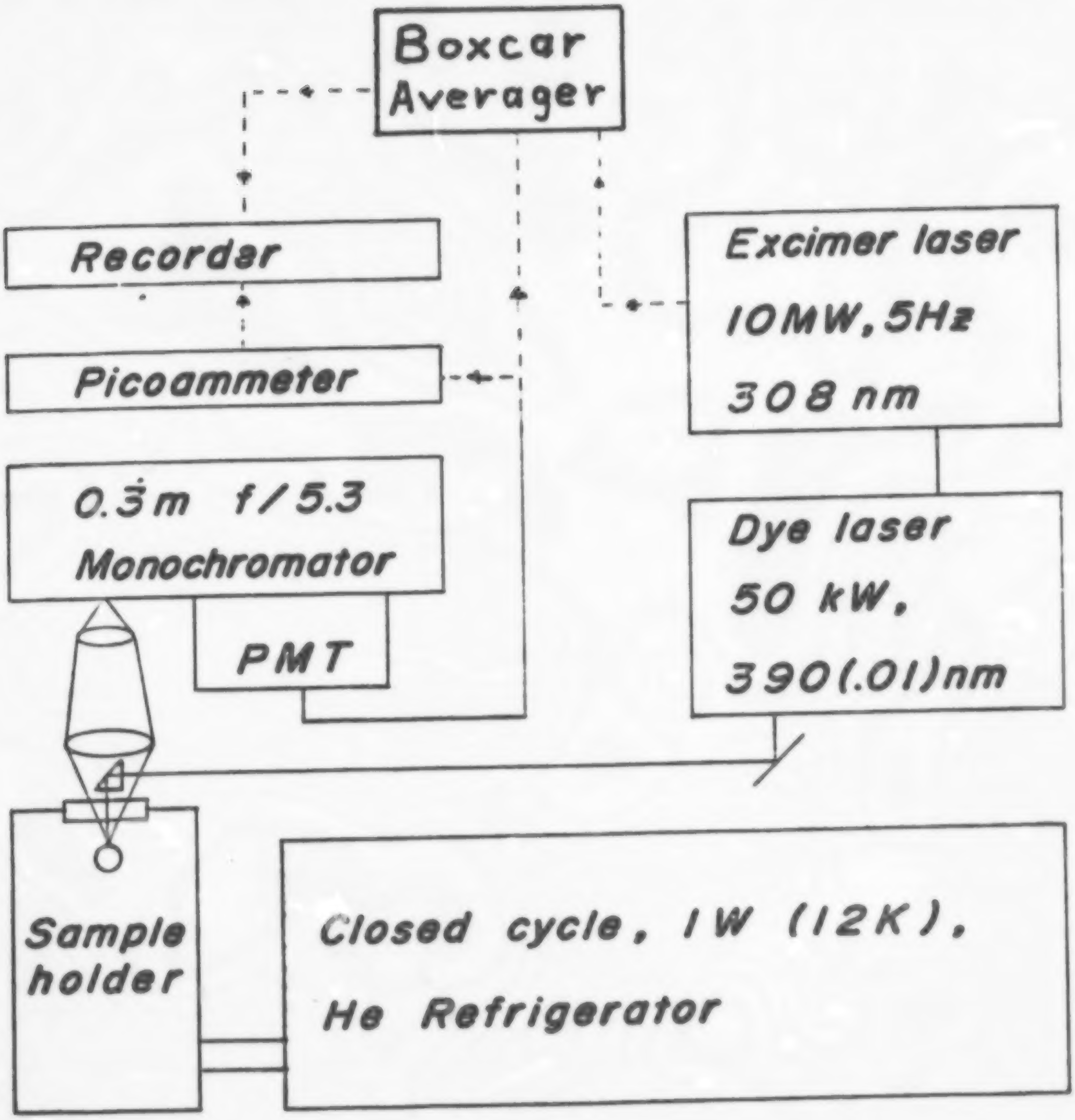

Boxcar Averager
Recorder
Picoammeter
0.3m f/5.3 Monochromator
PMT
Excimer laser
10MW, 5Hz
308 nm
Dye laser
50 kW,
390(.01)nm
Sample holder
Closed cycle, 1W (12K),
He Refrigerator

Figure 4 Equipment Schematic

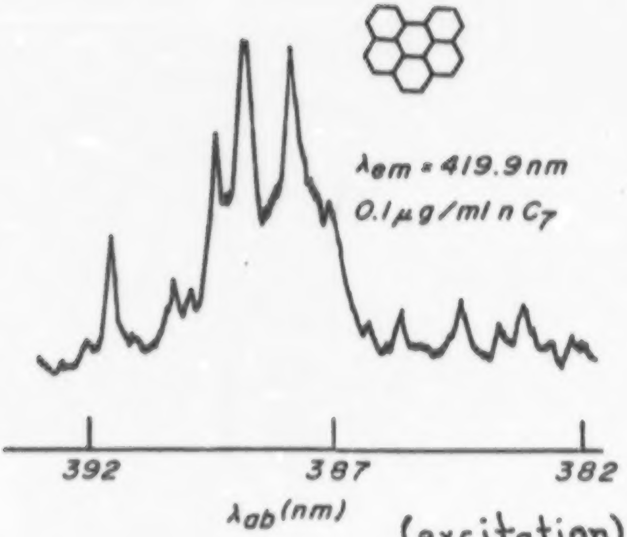
into a Dewar of liquid nitrogen to 'quick-freeze' it before insertion into the copper block which was at a temperature of 77°K. This procedure aided the formation of the polycrystalline structure needed for Shpol'skii spectroscopy (1,9).

A tunable dye laser (Lambda Physik model FL2000) pumped by an excimer laser (Lumonics model 260-2) was used as an excitation source (see figure 4). The dye laser radiation (0.01 nm. bandwidth) could be tuned from 400 nm. to 360 nm. Fluorescence or phosphorescence from the sample was collected by a lens and focussed onto the slit of a monochromator (McPherson, model 218). Dispersed radiation was detected by a cooled photomultiplier tube (EMI, model 6256S) and then directed to the electronic processing equipment. Either box car averaging (using Evans Associates modules 4141 and 4130) or an electrometer (Keithley model 610B) with R-C averaging was used to convert the current pulses into a voltage suitable for a chart recorder. Excitation specta (i.e. absorption spectra) were obtained by fixing the monochromator at a known fluorescence wavelength, and then recording the intensity of that fluorescence as the dye laser was tuned through its range. Fluorescence spectra were obtained by selecting a fixed excitation wavelength and then recording the output of the photomultiplier as the monochromator was scanned from 390 nm to longer wavelengths. An optical cut-off filter was used in front of the monochromator to reduce the amount of scattered laser light reaching the photomultiplier tube.

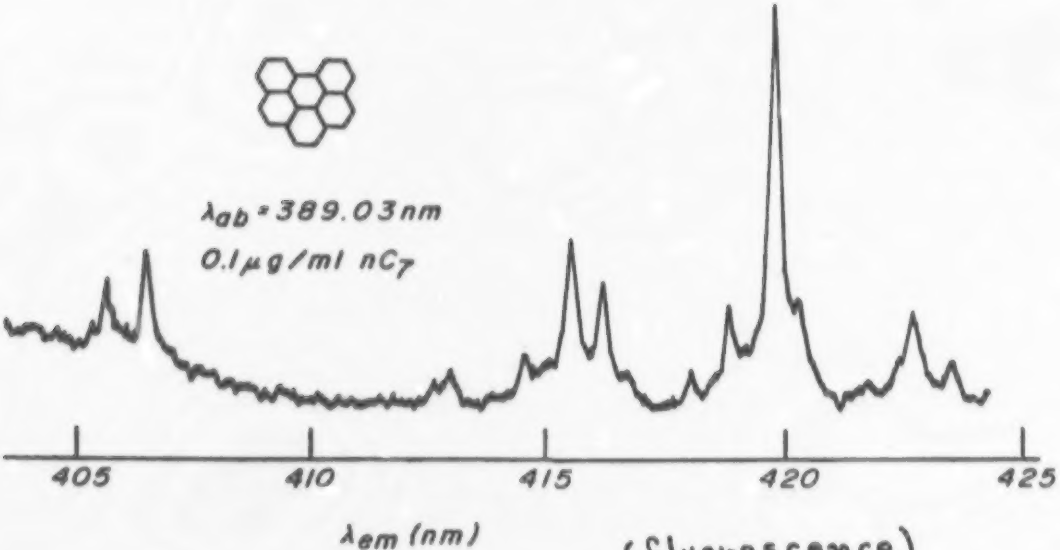
RESULTS

We have recorded many spectra under various conditions of laser energy, compound concentration, spectral range, sample cooling rate, and electronic signal processing. For discussion we have selected a small number of spectra to illustrate both the technique, and our progress towards the analysis of an environmental sample. Included are both excitation spectra (360 nm to 400 nm) and fluorescence spectra (390 nm to 500 nm). The former are analagous to absorption spectra, whereas the latter are emission spectra from the first excited singlet state of the molecule. As yet, we have not studied phosphorescence emission, i.e. radiation from the triplet state. None of the spectra have been corrected for temporal or spectral variations in laser energy, nor for spectral variations in the detector response.

Figures 5 and 6 show excitation and fluorescence spectra of benzo (ghi) perylene, and perylene, while figure 7 shows two excitation spectra of perylene which were recorded at different fluorescence wavelengths, 445 nm and 478.5 nm. Figure 8 shows two fluorescence spectra of pentacene obtained at two different excitation wavelengths, 369.71 nm and 389.96 nm. Spectra of benzo (ghi) perylene at three different concentrations are shown in figure 9. In figure 10 are shown the excitation and fluorescence spectra of benzo(a) napthacene in our standard excitation and fluorescence ranges, while figure 11 shows two excitation spectra from a sample of benzo(g) chrysene. Lastly figure 12 has two spectra of an environmental sample from Hamilton, Ontario. One of the spectra was obtained from a dilute solution in methanol, whereas the other came from a solution in heptane. A more complete discussion of these results follows.



(excitation)



(fluorescence)

Figure 5 Benzo(ghi) perylene

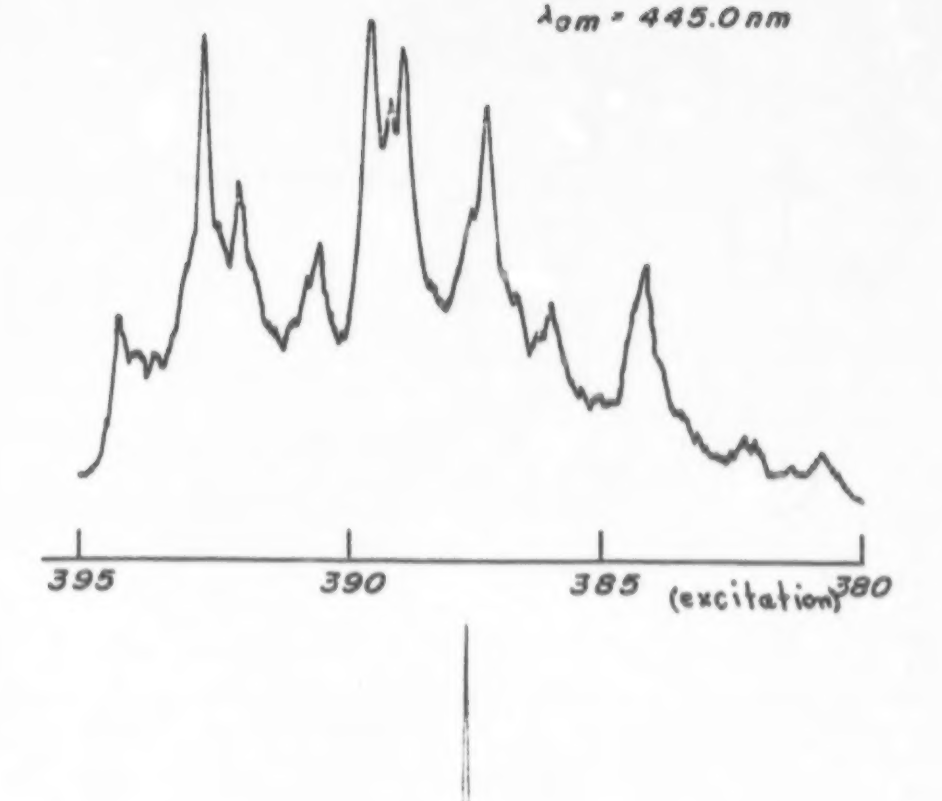


Figure 6 Perylene

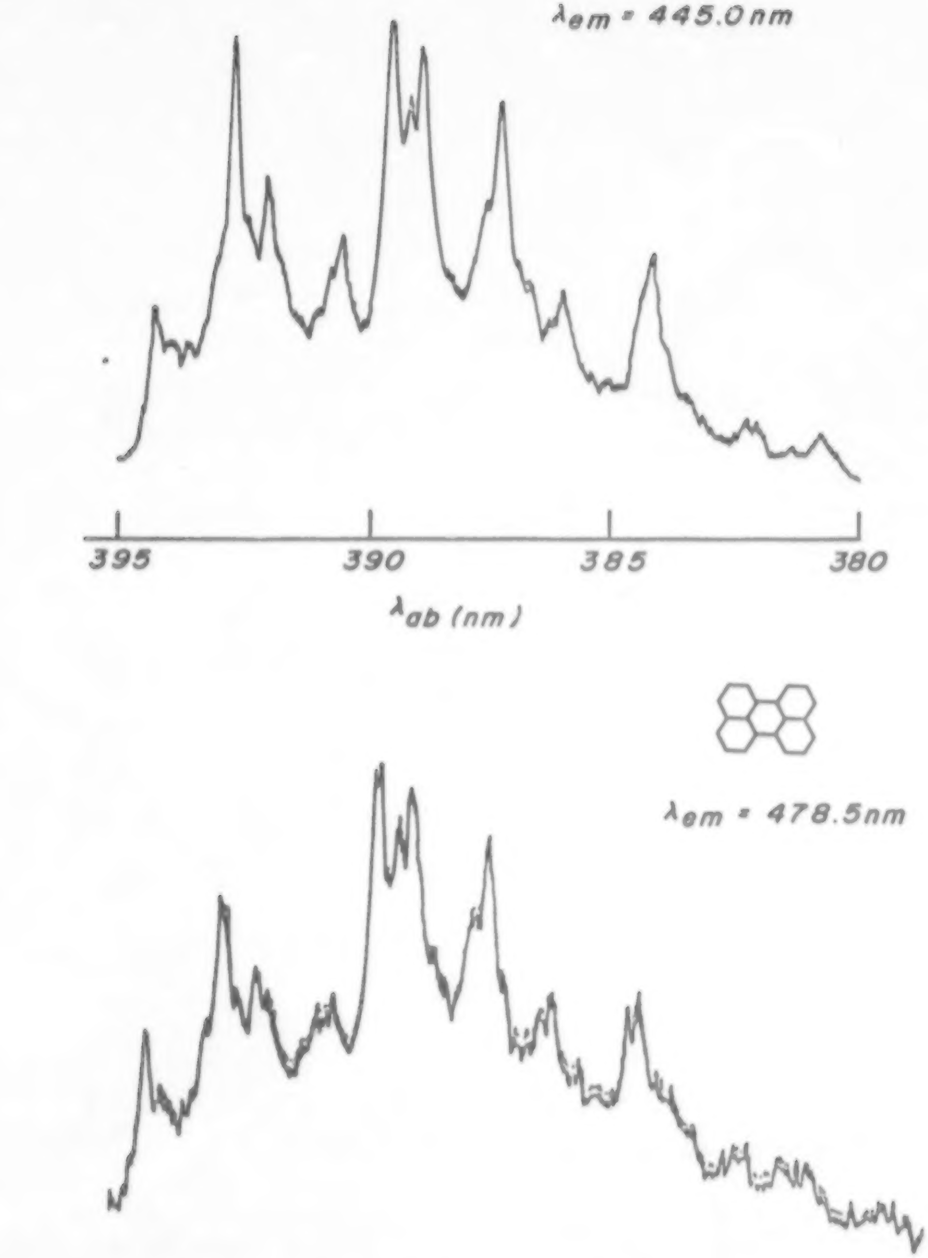


Figure 7 Excitation Spectra

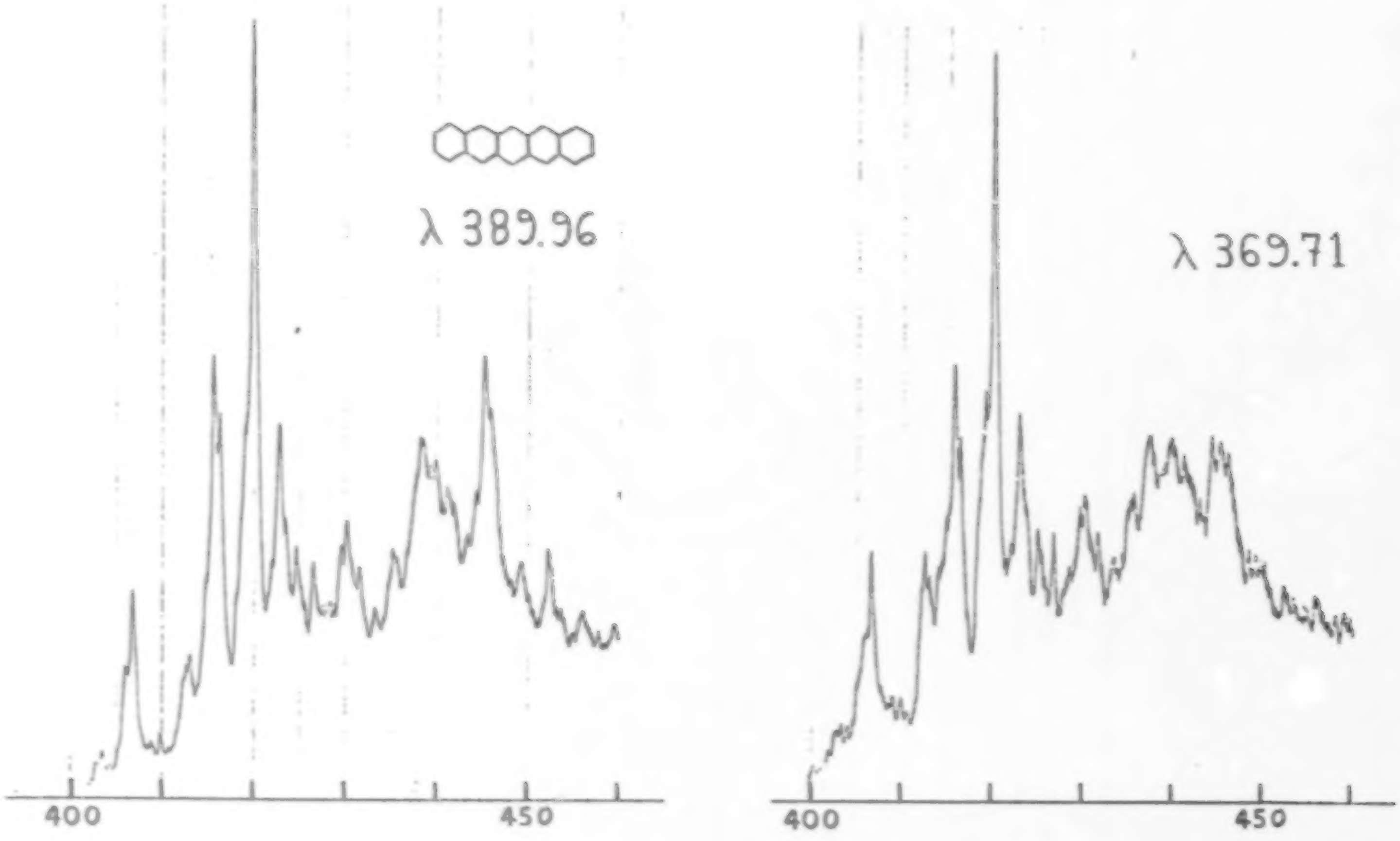


Figure 8 Fluorescence Spectra

DISCUSSION

The fluorescence spectra of benzo(ghi) perylene, and of perylene are clearly well separated from one another (figs. 5,6). Both compounds were on the list of six priority pollutants issued by the International Agency for Research on Cancer in the early 1970's. Their excitation spectra clearly show regions of overlap where a single excitation wavelength would jointly excite well separated fluorescence spectra from both compounds. Equally there are obvious locations where little overlap occurs and one compound's fluorescence spectrum could be preferentially excited. Both possibilities suggest approaches which might be useful in the analysis of these two compounds.

Figures 7 and 8 illustrate the implications of the Kasha rule for the spectra of PAH compounds in a Shpol'skii matrix. In figure 7 we see two excitation spectra of perylene, each of which closely resembles the other although the fluorescence wavelengths at which these absorption spectra were recorded differ substantially from one another. According to the Kasha model this behaviour can be expected since the appearance of any particular spectral feature will not depend on the wavelength of excitation although its intensity will. This is clearly evident from the two spectra in figure 7. Similarly, figure 8 shows two fluorescence spectra of pentacene which were excited at two very different wavelengths. These two spectra are essentially identical as we would expect on the basis of the Kasha model.

Quantitative determination of the amount of a PAH compound present in a sample is important. Figure 9 shows spectra from benzo(ghi) perylene obtained at three different concentration levels. Although the vertical scale is arbitrary there is a linear relationship in the relative intensities of the most intense feature over this concentration range. Moreover, it is clear that the intensity of the most intense feature is still well above the background level at the lowest concentration, and we estimate that we ought to be able to obtain a useful spectrum for at least another order of magnitude reduction in the concentration. The background level is the major limitation on the absolute sensitivity of this method. We are currently examining the contributions of photomultiplier dark current and of phonon emission (11) to this background. Additionally, these three spectra indicate the effect of concentration on the sharpness of the quasi-lines from the Shpol'skii effect. The spectrum taken at the highest concentration ($\sim 4\times10^{-5}$M.) shows broader features than do the other two. This is an example of an empirical result, likely connected with some sort of clumping of the solute, that indicates concentrations above 10^{-5}M. should be avoided.

The excitation and emission spectra from benzo(a) napthacene shown in figure 10 may seem puzzling on first glance. Little structure is present in the excitation spectrum whereas the fluorescence spectrum does have structure. Yet the "mirror image" phenomenon mentioned previously suggests that structure in the fluorescence spectrum ought to be reflected as structure in the excitation spectrum (and vice versa). Two possibilities exist. One, the excitation region chosen for the fluorescence feature is poorly suited for effective excitation of this compound. Indeed, there is a substantial gap between the onset of fluorescence at 450 nm and the longest wavelength for excitation, which is 400 nm. Unfortunately, the dye in our tunable dye laser was inefficient at wavelengths longer than 400 nm. In addition scattered laser light would be a substantial problem at excitation wavelengths longer than 400 nm. As a result we were not able to explore this point. A second possibility is that the molecule does not display a "mirror image" behaviour. Such molecules are known.

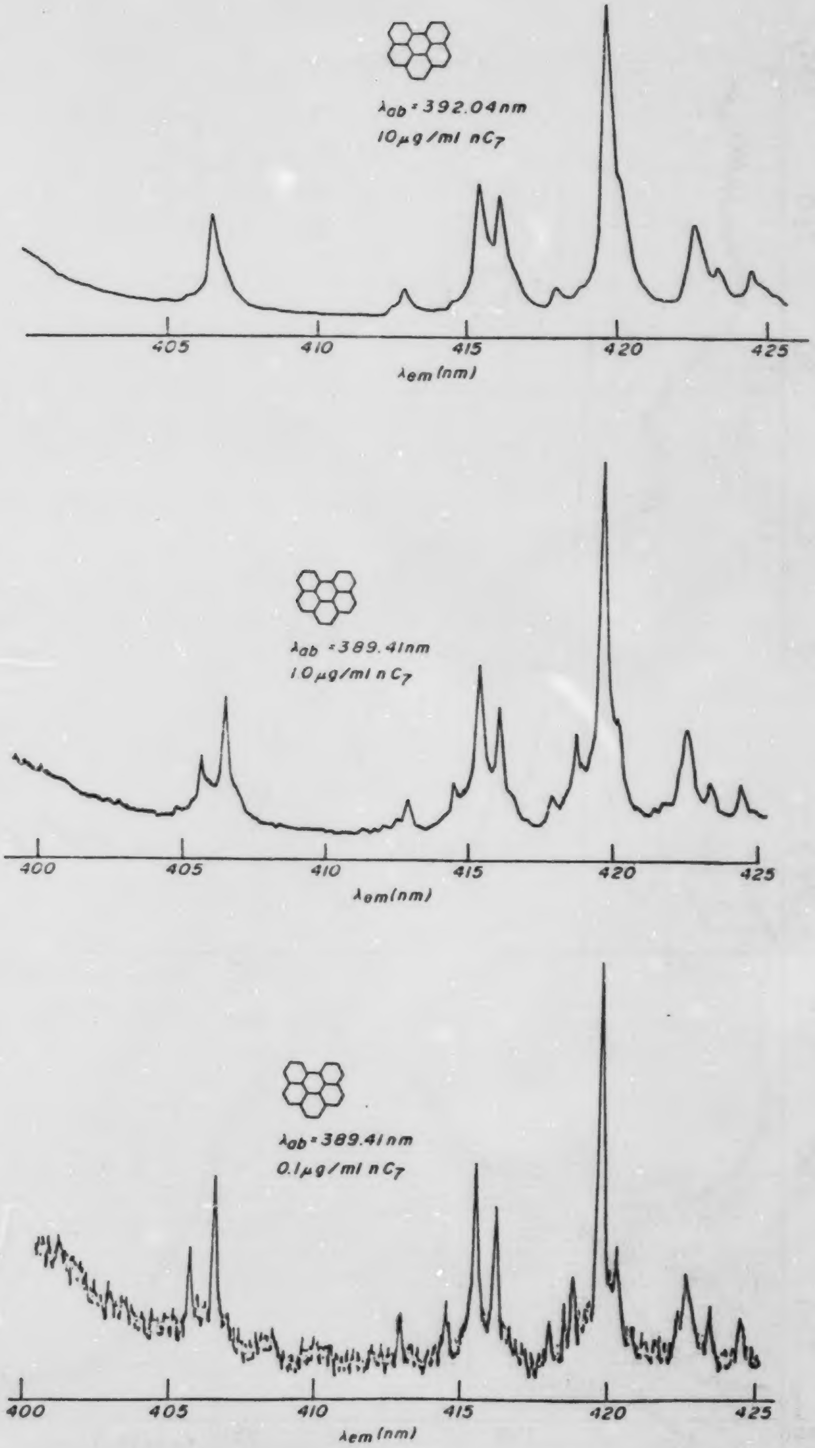


Figure 9 Spectra at 3 dilutions

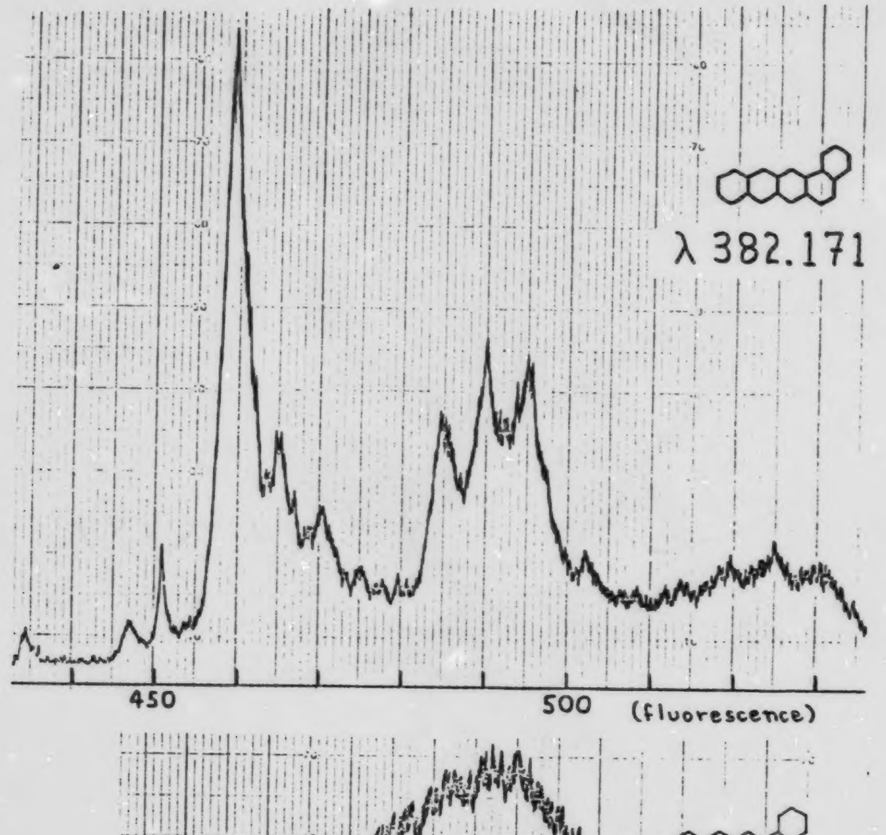


Figure 10 Benzo(a)napthacene

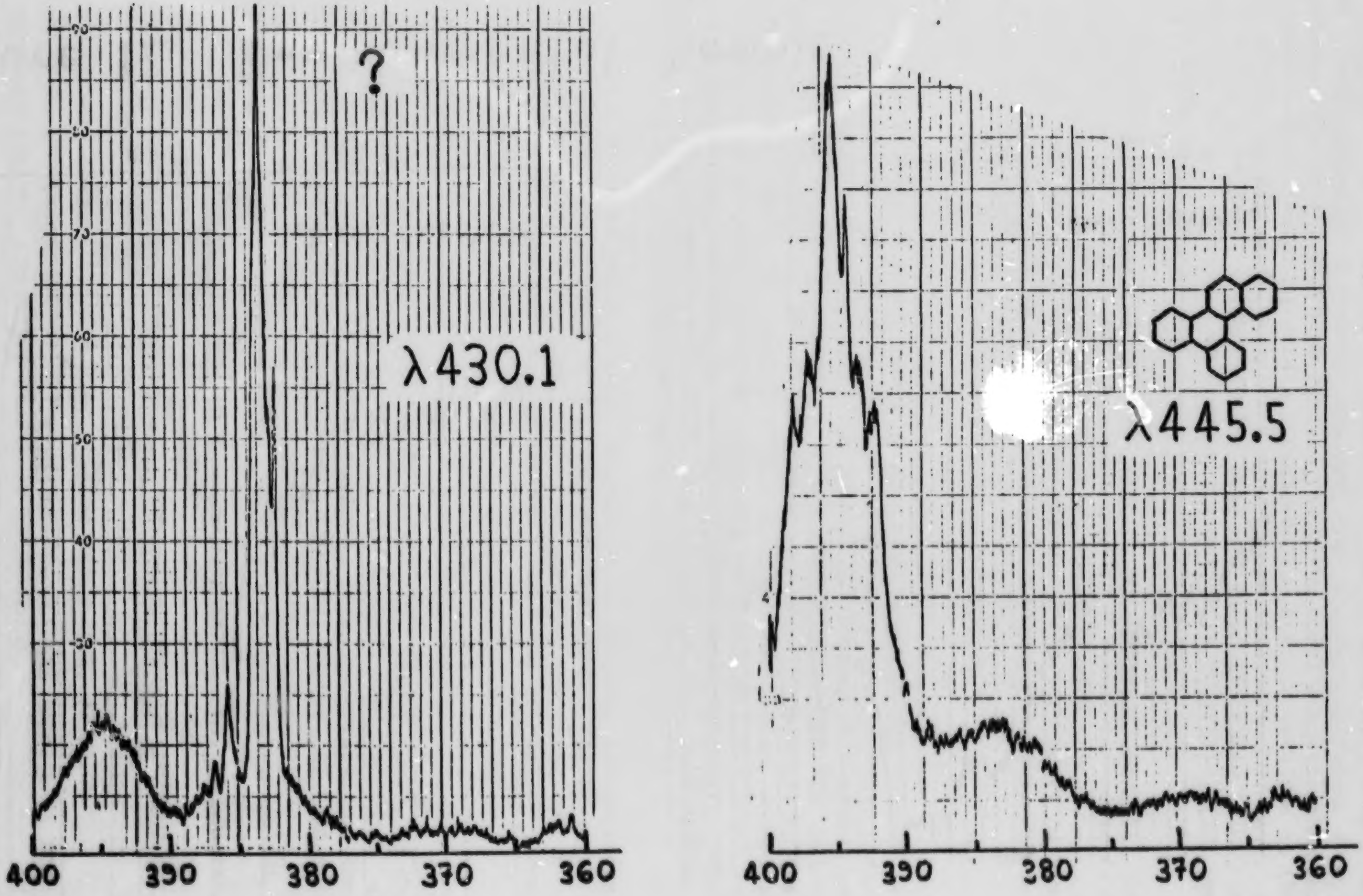


Figure 11 Excitation Spectra from Same Sample

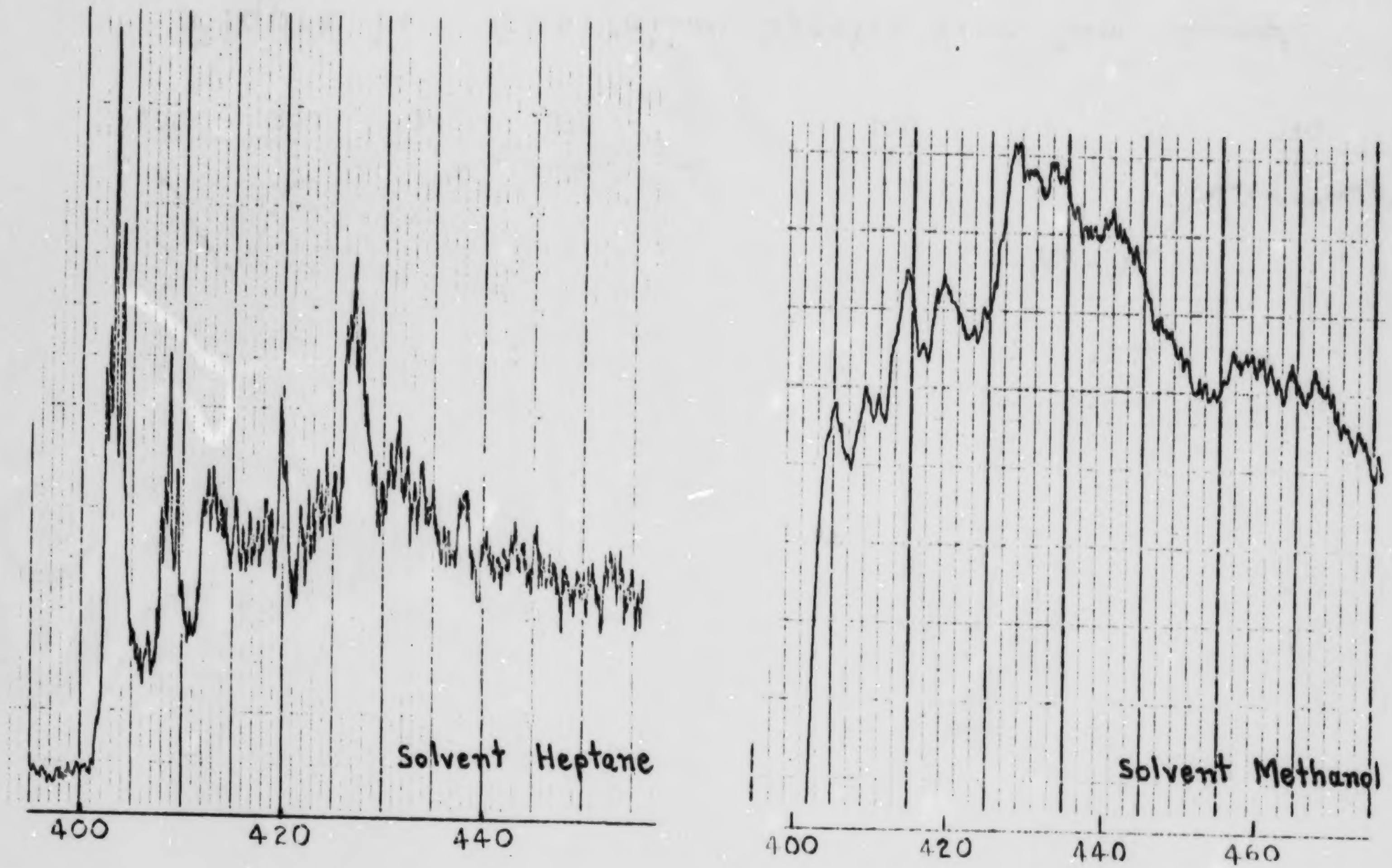


Figure 12 Environmental Sample

The two excitation spectra shown in figure 11 ostensibly come from the same sample, benzo(g) chrysene. However, they are very different even though they cover the same wavelength range. The Kasha model suggests they must then represent two different materials. Fluorescence spectra obtained at the major peak of the two different excitation spectra are also very different. Although we have not been able to identify the compound shown in figure 11(b), it clearly appears to be an impurity. At present its origin is uncertain. The usefulness of the Kasha model is apparent in this instance.

Figure 12 shows spectra obtained from an environmental sample. The spectrum, 12(b), in which the sample was dissolved in methanol has broad features with few sharp 'quasi-lines'. By contrast, that obtained with normal heptane as the solvent has many sharp features, several of which are attributed to benzo(a) pyrene. We are currently in the process of identifying the other PAH compounds present in this sample.

SUMMARY

An analytical procedure based on Shpolskii spectroscopy appears to be well suited to the detection of polycyclic aromatic hydrocarbons, their derivative compounds, and their heterocyclic analogues. Efforts at this method and integrating it into the standard repertoire of analytical techniques are well advanced in France (12), Sweden (4), and the United States (5). Proper use of the method will require a library of comparison fluorescence, excitation, and probably phosphorescence spectra. Additional spectra obtained with different solvents may be a necessity. Tunable dye lasers provide valuable excitation sources for PAH spectra because they are intense, have narrow spectral band widths, and can be pulsed for use in time delayed spectroscopy.

We have begun to build a small collection of spectra, concentrating on the six priority pollutants named by the International Agency for Research on Cancer; and we have begun to confront the problem of applying the technique to an environmental sample of unknown composition, which may consist of a complex mixture of PAH's and other compounds.

ACKNOWLEDGEMENTS

We thank Dr. Snieckus (Univ. of Waterloo), Dr. Harvey (Univ. of Chicago), and Dr. Wise (U.S. Nat. Bureau of Stand.) for several PAH compounds. Also we thank the Ontario Ministry of the Environment, York University, and the Natural Sciences and Engineering Research Council for their support of this work.

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SAMPLING AND ANALYSIS OF PAH DERIVATIVES IN URBAN AIR PARTICULATES

M. A. Quilliam, P. D'Agostino and J. Marr
Department of Chemistry, McMaster University, Hamilton, Ontario

INTRODUCTION

The chemical composition of urban air particulate matter is very complex, consisting of hundreds of organic and inorganic components. Over the last decade, many efforts have been directed to the determination of the levels of known carcinogenic compounds, particularly polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene, in the organic solvent extractable fraction. However, in recent years it has become clear from work with microbial mutagenesis assays and chemical fractionation techniques that there are many hazardous substances present that are still largely uncharacterized. Studies have shown that much of the mutagenic activity in air particulate extracts is associated with compound classes other than the PAHs and that much of the activity is of the "direct-acting" type (1). The term "direct-acting" applies to those substances that cause reversion of Salmonella test strains in the Ames microbiological reversion assay (2) in the absence of the microsomal fraction of rat liver. The latter provides the oxidative "activation" of many chemicals to the reactive intermediates that act as the ultimate mutagens or carcinogens.

Several studies have shown that nitrated and oxygenated polycyclic aromatic hydrocarbons (nitro-PAHs and oxy-PAHs) are probably the principle direct-acting mutagens in automobile exhaust and urban air particulate samples (3,4). The environmental significance of these mutagens is not yet fully established, however, due in part to the lack of analytical methods for their determination and in part to the fact that many of these compounds may be formed as artifacts during the sampling step. Recent studies have shown that some PAHs can react readily with filter media, nitrogen oxides, sulfur oxides, ozone, and gas-phase photochemical smog to form a variety of derivatives (5).

Our research group has been examining the environmental and toxicological significance of the nitro-PAHs. Out of this work we have developed a routine analytical method that is sensitive and selective for the determination of nitro-PAHs in environmental samples, such as diesel exhaust and urban air particulates (6). The key steps in the procedure are: (a) a clean-up scheme that isolates the nitro-PAHs from the other components in the sample, (b) screening and quantitation by capillary column gas chromatography with simultaneous flame ionization and nitrogen-selective detection, and (c) structural confirmation with gas chromatography-mass spectrometry. In addition we have synthesized a large number of standard compounds that have been characterized by retention indices, mass spectra, and UV-VIS and NMR spectra.

Currently, we are extending our clean-up method to a wider range of compounds, investigating the extent to which nitro-PAHs and other PAH derivatives are artifacts of the sampling procedure, and studying new methods of sampling. The latter work is being done in collaboration with researchers at Concorde Scientific who are presently involved with the Ministry in contract research on alternatives to hi-vol sampling for PAHs. This paper will detail the analytical methodology that we have developed and the talk will present our latest results.

METHODS

Methylene chloride, acetone and methanol were "distilled in glass" quality purchased from Caledon Laboratories Ltd. (Georgetown, Ontario). Hexane and isopropanol were "HPLC grade" purchased from BDH (Toronto, Ontario). Nitro-PAHs were prepared from their corresponding PAHs by treatment with fuming nitric acid in acetic anhydride at 25°C. Depending on the reactivity of the PAH, the amount of fuming nitric acid used had to be varied in order to get predominantly mono-nitration or di-nitration. [^{3}H]-1-Nitropyrene was prepared as described previously (7).

Diesel particulate samples were provided by Dr. I. Salmeen of Ford Motor Co., Detroit. Urban air particulate samples from the Hamilton area were provided by Dr. L. D. Pengally (McMaster University, Hamilton, Ontario). Samples were collected for 24 hours at an average flow rate of 47 cubic feet per minute on 8 X 10 inch glass fiber filters (Schleicher and Schull Inc., Keene, N.H.) using a high volume total suspended particulate sampler (General Metal Works, Clev., Ohio). Air particulate samples were extracted with methylene chloride overnight in a Soxhlet extractor. Following concentration to about 10 ml in a rotary evaporator, the organic extract was filtered to remove particulates (0.5 um teflon filter, Millipore Corp., Bedford, MA) and evaporated to dryness under a stream of nitrogen. The weight of the dried residue was then determined.

The extracted organics dissolved in 1 ml of CH_2Cl_2 were injected into a silica Sep-Pak (Waters Associates Inc., Milford, MA) that had been previously washed with 5 ml CH_2Cl_2. With CH_2Cl_2 used as the eluent, the first ml of eluent was discarded while the next 3.5 ml was collected and evaporated to 200-300 ul under a stream of nitrogen. This solution was injected directly onto a Sephadex LH-20 column (Pharmacia Fine Chemicals, Dorval, Quebec) previously equilibrated in and eluted with methanol/methylene chloride (3:1). The eluent was monitored at 254 nm. The aromatic fraction which eluted in the 15-25 ml range was collected and evaporated to dryness under a stream of nitrogen.

In the final clean-up step a normal phase semi-preparative HPLC with a 250 X 9.4 mm M9 PAC column (Whatman Inc., Clifton, NJ) with a hexane/isopropanol mobile phase was used. The sample was dissolved in 20 ul methylene chloride and 80 ul hexane prior to injection. Excessive amounts of methylene chloride could not be used since the separation of the PAHs from the nitro-PAHs is impaired. Both the PAH and nitro-PAH fractions were eluted from the M9 PAC column using 0.5% isopropanol/hexane at a flow rate of 6 ml/min. PAHs were collected in the 5.0 to 8.8 min range and nitro-PAHs in the 8.8 to 18.0 min range. The nitro-PAH fraction could be further split into 2 to 3 ring nitro-PAHs (8.8 to 12.5 min) and 4 to 5 ring nitro-PAHs (12.5 to 18.0 min). Dinitro-PAHs and more polar materials may be eluted using a step gradient from 0.5% to 10% isopropanol/hexane at the 20 min mark and collecting from 18 to 35 min. Fractions were evaporated to dryness and dissolved in a known volume of acetone for the final GC analysis.

A Varian 3700 gas chromatograph equipped with a cold on-column injector and flame ionization (FID) and nitrogen-phosphorus (NPD) detectors was used for GC analyses. An effluent splitter provided simultaneous FID and NPD detection. Two fused silica capillary columns were used: a) 25 m X 0.31 mm I.D., 0.17 um OV-1 (Hewlett-Packard, Mississuaga, Ontario), and b) 15 m X 0.32 mm I.D., 0.1 um DB-5 (JW Scientific Inc., Orangevale, CA). All analyses were performed with a temperature program of 60° to 150° at 14°/min, followed by 4°/min to 320°.
An Apple II+ microcomputer (Apple Computer Inc., Cupertino, CA) equipped with a 12-bit analog-to-digital converter (Interactive Microware Inc., State College, PA) was used for data aquisition, storage and plotting. HPLC was performed on Spectra-Physics 8000 liquid chromatograph (Santa Clara, CA) equipped with a Beckman 153 UV detector (Irvine, CA).

GC-MS analyses were performed on a VG Micromass 7070F mass spectrometer (VG Analytical, Altrinchan, U.K.) equipped with a Varian 3700 gas chromatograph. Samples were chromatographed on the fused silica capillary columns described above with the same temperature program. The column was connected directly to the mass spectrometer and inserted to within 2 cm of the electron beam. Electron impact ionization with an electron energy of 70 eV was used. Data was acquired with a VG 2035 data system, either by scanning from 400 to 80 amu, at 1 sec/decade, or by performing selected ion monitoring on up to 7 ions with accelerating voltage stepping and a 100 msec sampling interval for each ion.

RESULTS AND DISCUSSION

In the analysis of a specific group of compounds present at trace levels in complex environmental samples, an analytical clean-up scheme directed toward the isolation of these compounds can dramatically reduce interferences in the final analytical step. The use of very specific, sophisticated methods of analysis such as MS/MS, while very powerful, are not readily available to most analysts and are not usually suitable to routine analysis. Since nitro-PAHs are found at trace levels in environmental samples an efficient clean-up scheme capable of routinely isolating this compound class from the complex matrix would be beneficial. For these reasons we have developed a specific clean-up scheme to isolate nitro-PAHs from diesel exhaust and urban airborne particulate matter (see Figure 1).

Airborne particulate samples, collected on glass fiber filters were Soxhlet extracted in methylene chloride overnight. Ultrasonic extraction with methylene chloride was investigated but was found to yield only about 10% of the total weight of extractable organics. Following evaporation the methylene chloride extract was

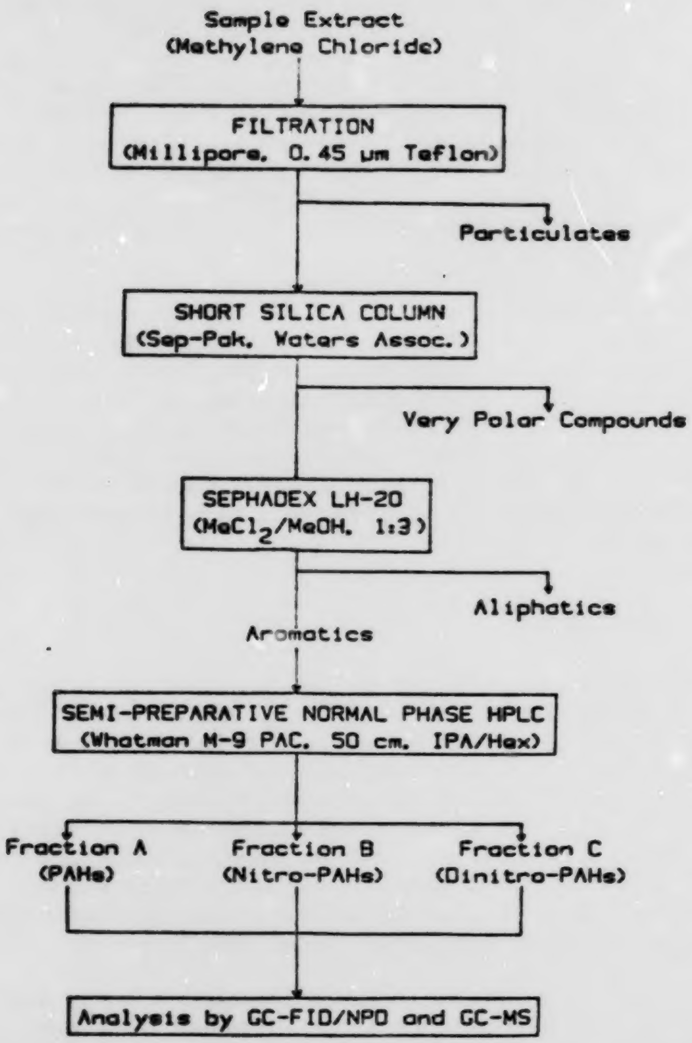
Sample Extract
(Methylene Chloride)
FILTRATION
(Millipore, 0.45 um Teflon)
Particulates
SHORT SILICA COLUMN
(Sep-Pak, Waters Assoc.)
Very Polar Compounds
SEPHADEX LH-20
(MeCl2/MeOH, 1:3)
Aliphatics
Aromatics
SEMI-PREPARATIVE NORMAL PHASE HPLC
(Whatman M-9 PAC, 50 cm, IPA/Hex)
Fraction A
(PAHs)
Fraction B
(Nitro-PAHs)
Fraction C
(Dinitro-PAHs)
Analysis by GC-FID/NPD and GC-MS

Figure 1: Clean-up scheme developed for the isolation of PAHs and nitro-PAHs in environmental samples.

filtered through a silica Sep-Pac to remove traces of particulates and highly polar compounds prior to the chromatographic steps where such compounds could interfere. In particular, with respect to the normal phase HPLC step, these compounds could lengthen analysis time, do irreversible damage to the column or cause memory effects.

The next step in the clean-up scheme was a class separation of aliphatics and aromatics using a Sephadex LH-20 column. A mobile phase of methylene chloride/methanol (1:3) was found to elute the aromatic fraction (PAHs, nitro-PAHs and other derivatives) as a relatively narrow band suitable for collection and concentration. The necessity of including this step was illustrated by its omission in the analysis of one air particulate sample. The resulting gas chromatogram was considerably more complicated, particularly with the presence of other nitrogen-containing compounds.

The final clean-up step involves normal phase HPLC, using a semi-preparative column with a mixed amino-cyano bonded phase, to separate nitro-PAHs from PAHs (see Figure 2). This is a desirable separation step because the PAHs are in far greater abundance than the nitro-PAHs and would interfere in both GC and GC-MS analyses. The advantages of using HPLC with a chemically bonded phase column rather than a conventional silica or alumina column include better reproducibility, higher resolution and less chance for irreversible absorption of trace components. A number of normal phase columns were investigated and it was found that the Whatman PAC (mixed amino-cyano) column afforded the best separation of PAHs and nitro-PAHs with a mobile phase of 0.5% isopropanol/hexane. If desired, the nitro-PAH fraction could be split according to ring size (e.g., 2-3 rings and 4-5 rings). In addition, it is possible to isolate another fraction containing dinitro-PAHs.

The clean-up scheme was tested for efficiency with tritiated 1-nitropyrene in the presence varying amounts of cold carrier, as well as sample matrix. An average recovery of 85% was observed over the nanogram to microgram range. This was considered to be adequate for trace analysis work. Typical front end loading for the clean-up scheme was in the order of 50 to 70 mg of extractable organics. It is estimated that the maximum capacity of the scheme as presented is at least twice this amount. The major problem is the exceedingly low solubility of the nitro-PAHs and the limited injection volume sizes that must be used to prevent deterioration of chromatographic efficiency. Larger column sizes would allow a greater capacity for the procedure.

A number of analytical finish techniques techniques have been evaluated. Reverse phase HPLC analysis with a UV detector was found to have considerable interference even after our clean-up, and confirmation of peak identity by mass spectrometry is difficult compared to GC-MS. We have found that GC-MS with selected ion monitoring, especially in the negative ion chemical ionization mode, offers very high sensitivity and selectivity for nitro-PAHs. However, it was not possible to justify dedication of our mass spectrometer to routine monitoring. We concluded therefore that capillary column gas chromatography with selective detection was the method best suited to routine screening of samples. Both electron capture and

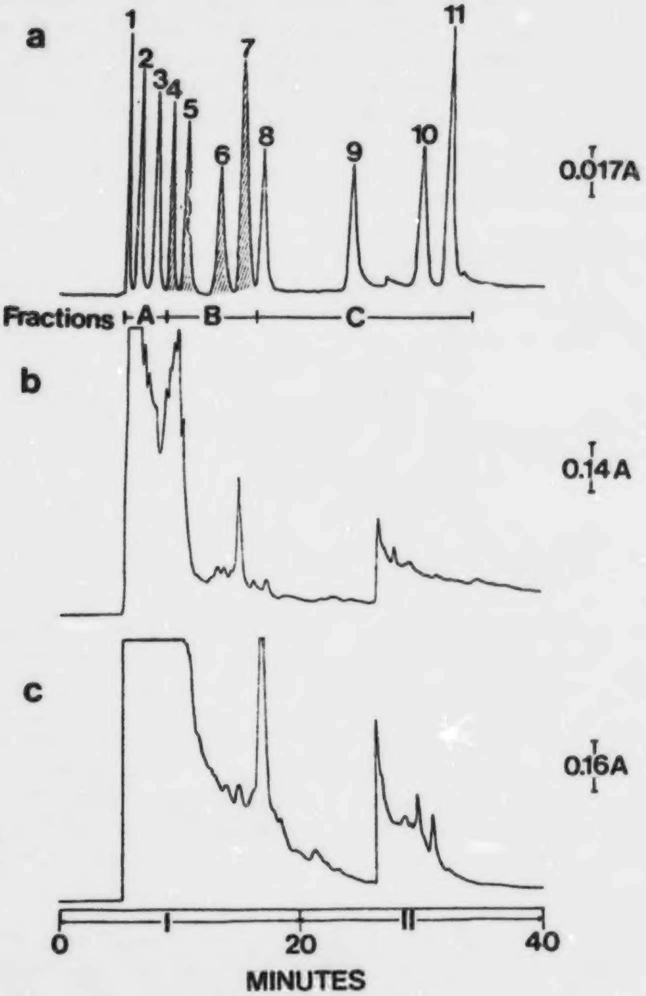

Figure 2: HPLC semi-preparative chromatography used in the clean-up scheme. Chromatogram (a) illustrates the separation of a mixture of standards (1 = naphthalene; 2 = pyrene; 3 = benzo[a]pyrene; 4 = 2-nitronaphthalene; 5 = 9-nitroanthracene; 6 = 1-nitropyrene; 7 = 6-nitrobenzo[a]pryvene; 8 = 1,5-dinitronaphthalene; 9, 10 and 11 = 1,3-, 1,6- and 1,8-dinitropyrene). The other chromatograms illustrate the separation of organics isolated from the Sep-Pak and Sephadex LH-20 clean-up for: (b) a Peugot diesel exhaust particulate sample, and (c) a Toronto airborne particulate sample. See Methods for the conditions.

thermionic sensitive (nitrogen-phosphorous, NPD) detectors were investigated and it was determined that nitrogen selective detection provided the greatest selectivity.

The results that we report here were acquired with simultaneous flame ionization/nitrogen selective detection (FID/NPD). This method provides information on other components in the fractions analyzed and, in addition, allows accurate measurement of retention indices through co-injection of n-alkane standards. The use of cold on-column injection and fused silica columns was found to be essential for quantitation at trace levels. Flash injection techniques or packed columns showed poor detection limits for nitro-PAHs. Our GC-NPD analysis shows a detection limit of approximately 40 pg and linearity up to 50 ng for standards.

Retention indices for a large number of synthetic nitro-PAH standards have been measured. Many PAHs give several isomeric nitro-PAHs when nitrated, but the positions of substitution have been determined for only a few. Work in this area is continuing, as well as testing of the mutagenicity of different nitro-PAHs.

Some typical results for the analysis of nitro-PAH fractions of diesel exhaust and urban air particulate samples are illustrated in Figures 3 and 4, respectively. The identities of numbered peaks are given in Table 1. Nitro-PAHs were initially identified by retention indices and then confirmation was made by GC-MS. Only 1-nitropyrene was concentrated enough to give a good full electron-impact mass spectrum. The other nitro-PAHs were confirmed by selected monitoring of the molecular ions and M-30 fragment ions. Quantitation of these compounds was performed with the NPD detector, relative to an internal standard (1-nitronaphthalene). Other peaks appearing in the FID traces were identified by full electron-impact mass spectra to be oxygenated-PAHs and a phthalate. Although these cause interference in the FID trace, the NPD trace is not affected. The extent of clean-up is best illustrated by the fact that nitro-PAH peaks could not be observed in the chromatograms of the raw sample extracts prior to clean-up. They were masked by a large number of very intense peaks forming an "envelope" in the chromatogram. In addition, only one-hundredth of the material could be injected due to the presence of other compounds.

These methods are now being used to monitor the levels of PAHs, nitro-PAHs and oxy-PAHs in samples of airborne particulates that have been acquired with various types of sampling devices. The information from these experiments should be useful in determining the efficiency and degree of artifact formation with these different samplers.

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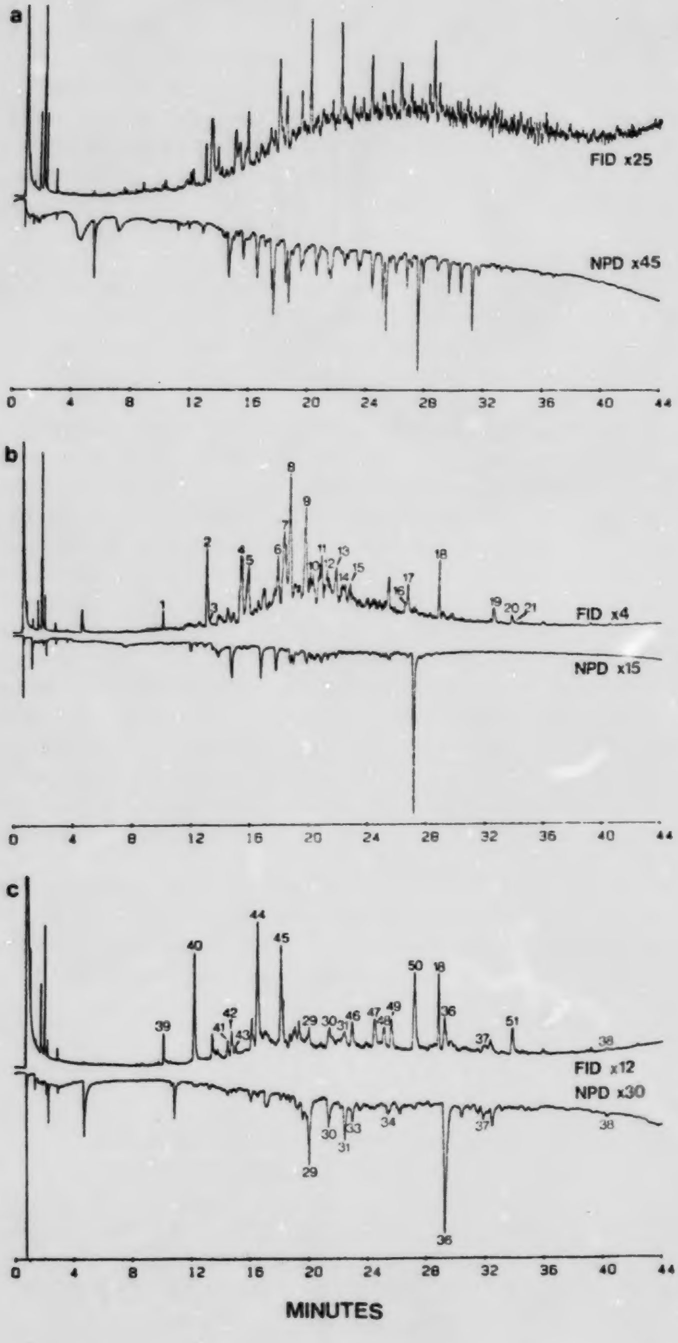


Figure 3: Capillary column GC analysis with simultaneous FID/NPD detection of an Oldsmobile diesel exhaust particulate sample. Chromatogram (a) is for the raw extract without clean-up, while chromatograms (b) and (c) are for the clean-up fractions A (PAHs) and B (nitro-PAHs), respectively. Column A (OV-1) was used with the conditions given in Methods.

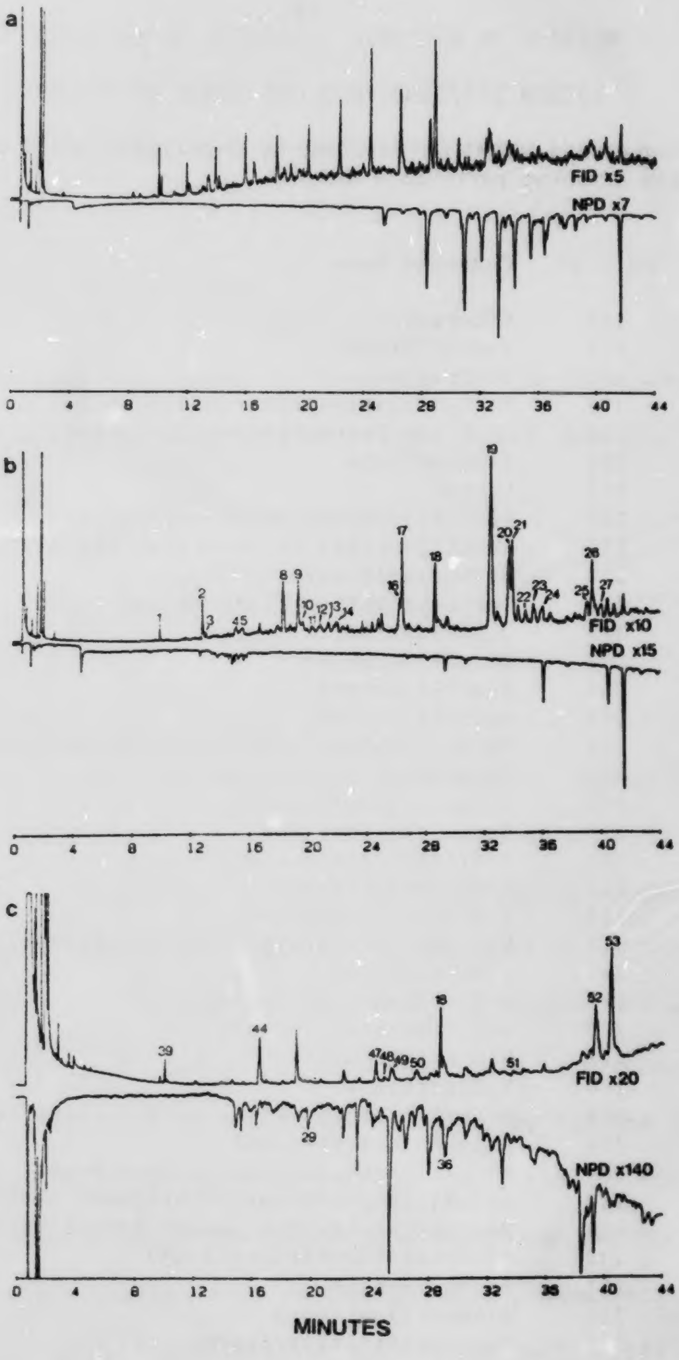


Figure 4: Capillary column GC analysis with simultaneous FID/NPD detection of a Hamilton airborne particulate sample. Chromatogram (a) is for the raw extract without clean-up, while chromatograms (b) and (c) are for the clean-up fractions A (PAHs) and B (nitro-PAHs), respectively. Column A (OV-1) was used with the conditions given in Methods.

Table 1: Compounds identified in clean-up fractions A and B of diesel exhaust and airborne particulate samples

Peak No.	Mol Wt	Compound Name
1	166	Fluorene
2	178	Phenanthrene
3	178	Anthracene
4,5	192	Methyl(phenanthrene/anthracene)
6,7	206	C2-Alkyl(phenanthrene/anthracene)
8	202	Fluoranthene
9	202	Pyrene
10,11,12	220	C3-Alkyl(phenanthrene/anthracene)
13,14,15	216	Benzofluorenes or Methyl(pyrene/fluoranthene)
16	228	Benz[a]anthracene
17	228	Chrysene and/or Triphenylene
18	390	Dioctylphthalate
19	252	Benzofluoranthene
20	252	Benzo[e]pyrene
21	252	Benzo[a]pyrene
22,23,24	266	Methyl(benzopyrenes/benzofluoranthenes)
25	276	Indeno[1,2,3cd]pyrene
26	278	Dibenz[ah]anthracene
27	276	Benzo[ghi]perylene
28	223	a-Nitrophenanthrene
29	223	9-Nitroanthracene
30	223	b-Nitrophenanthrene
31-35	237	Nitromethyl(phenanthrene/anthracene)
36	247	1-Nitropyrene
37	261	b-Nitrobenzo[a]fluorene
38	297	6-Nitrobenzo[a]pyrene
39	222	Diethylphthalate
40	180	9-Fluorenone
41,42,43	194	Methyl(fluorenone/phenanthracenone/anthracenone)
44	208	9 10-Anthracenedione
45	204	4H-Cyclopenta[def]phenanthen-4-one
46	220	Methyl(phenanthrene/anthracene)-carboxaldehyde
47,48,49	230	Benzoanthracenones and/or benzofluorenones
50	230	7H-Benz[de]anthracen-7-one
51	254	Benzopyrenone
52	280	Dibenzofluorenone
53	304	Cyclopenta[ghi]picenone

APPLICATION OF BENZAMIDE DIRECTED METALATION STRATEGY IN SHORT AND REGIOSPECIFIC ROUTES TO PERI-METHYL SUBSTITUTED PAHs

R.J. Mills and V. Snieckus*
Guelph-Waterloo Centre for Graduate Work in Chemistry
University of Waterloo, Waterloo, Canada N2L 3G1

INTRODUCTION

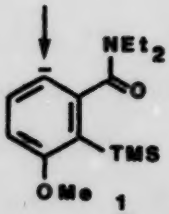
Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic pollutants which is widely dispersed in the environment. Recognition that this class of compounds plays a role in the epidemiology of occupationally-induced cancer has, in recent years, resulted in extensive research into the formation, occurrence, detection, chemistry and metabolism of PAHs. PAHs have been detected in the soil, in the atmosphere[1,2], in food[1,3], in water[4] and in tobacco and marijuana smoke[3,5]. Although there is evidence for the biosynthesis of PAHs by bacteria and higher plants[1], by far the major source of these environmental contaminants is from the combustion of fossil fuels.

Methylbenz[a]anthracenes (MBAs), PAHs that have been detected in cigarette smoke[6], stack gas and roofing tar extracts[7], have received much attention in studies concerned with structure-carcinogenic activity relationships[8]. Their presence in the environment emphasizes the need for the availability of synthetic samples, not only for their monitoring in the environment but also for continuing studies into the role that such PAHs play in the mechanism of carcinogenesis[9].

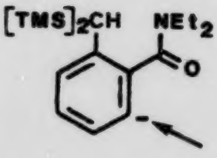
Apart from the extensive work of Newman[10], the availability of many of the monomethylbenz[a]anthracenes has been limited due to the complexity of the existing synthetic methods[11]. The evolving methodology of silicon protection in tertiary benzamide directed ortho lithiation[12] now allows its application to the synthesis of selected PAHs[13,14]. Details of this strategy[12] together with routes to selected peri-methyl substituted PAH precursors are herein reported.

RESULTS AND DISCUSSION

The regiospecific synthesis of polysubstituted benzene derivatives via the directed metalation of aromatic tertiary benzamides is well documented[15]. We have recently shown that the trimethylsilyl moiety can be used to effectively mask (a) the more reactive site to a carboxamide **1** and (b) the ortho methyl in an o-toluamide **2** to metalation reactions, thus allowing electrophile introduction into the alternate ortho positions (arrows in **1**,**2**) [12]. The ease of removal of the silyl groups[16] from the resulting products allows for rapid entry into diversely functionalized aromatic systems.



1

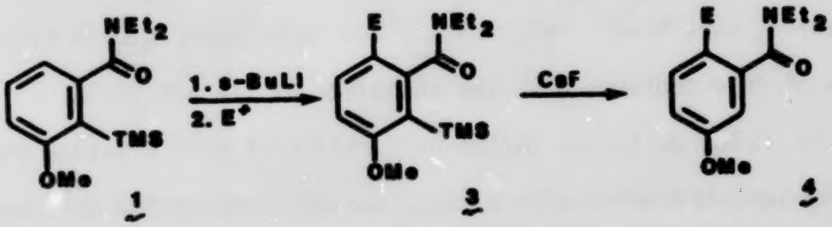


2

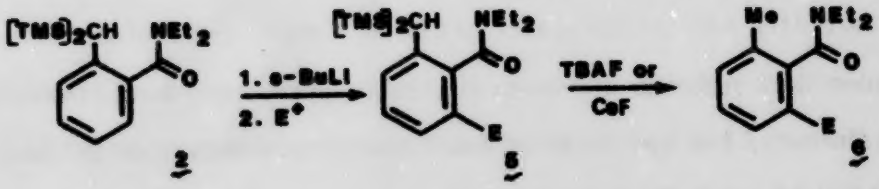
$TMS = SIMe_3$

Representative of the use of silicon protection of the preferred 2-site in attaining a 1,2,5-trisubstituted aromatic pattern is the lithiation of 1 (Scheme 1) under the standard conditions[17] followed by quenching with selected electrophiles leading to substituted benzamides 3 in excellent yields. Subsequent desilylation using CsF in DMF/H_2O (10:1) at reflux for 8-20 h affords functionally useful aromatics 4 in good yields[18] (E = CHO, CH_3, $CONEt_2$).

Scheme 1.

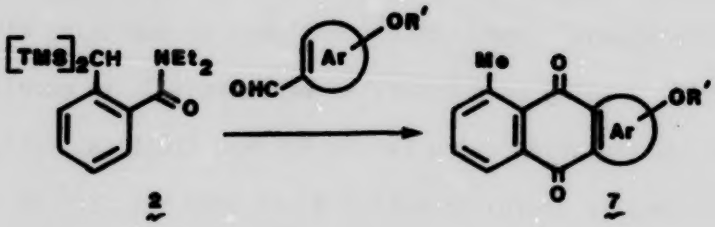


Scheme 2.



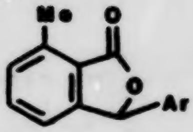
Compounds of the type 2 (Scheme 2) also undergo deprotonation under the standard conditions[17] to afford, after quenching with selected electrophiles, benzamides of the type 5 in excellent yields. Subsequent CsF or tetra n-butylammonium fluoride (TBAF)[16] mediated double desilylation affords 6-substituted-2-methyl benzamides 6 (E = $SiMe_3$, CH_3, CHO, SMe) in high yields. When the introduced electrophile is an aromatic aldehyde component, the silicon protection strategy in toluamide systems 2 allows for easy access to peri-methyl substituted PAH carcinogens[19] and naturally occurring anthraquinone natural products[20](Scheme 3).

Scheme 3.

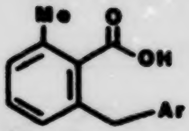


Thus metalation of the bis-trimethylsilyl *o*-toluamide 2[21] under the standard conditions[17] followed by sequential treatment with 2-naphthaldehyde, CsF(DMF-H_2O/reflux/9 h), and TsOH(PhMe/reflux/9 h) without purification of intermediates gave the phthalide **8a** in 52% yield. Hydrogenolysis ($CuSO_4$ activated Zn/10% NaOH/reflux/24 h)[22] provided the acid **9a**, which upon Friedel-Crafts cyclization (TFAA/HOAc/RT/9 h)[17] and oxidation ($Na_2Cr_2O_7$/HOAc/RT/9 h) afforded 11-methyl-7,12-benz[a]-anthraquinone **10a** in 31% overall yield from 2 . The conversion of **10a** into the highly carcinogenic 7,11,12-trimethylbenz[a]anthracene has previously been achieved[23].

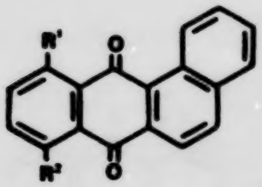
By an identical sequence of reactions, but substituting 1-naphthaldehyde for 2-naphthaldehyde, the synthesis of the intermediate phthalide **8b** and hence 8-methyl-7,12-benz[a]anthraquinone **10b** was effected in 42% overall yield from 2. Methods for the conversion of **10a** and **10b** into the corresponding PAHs are well documented[13]. The scope of this methodology was further exemplified by treating the lithiated species of 2 with 9-phenanthrene carboxaldehyde to afford, after subsequent desilylation and cyclization, the phthalide **8c** in 63% yield, an intermediate required for the preparation of 10-methyl-9,14-dibenz[a,c]anthraquinone **11**.



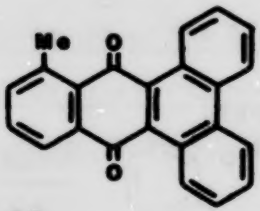
8a Ar=2-Naphthyl
8b Ar=1-Naphthyl
8c Ar=9-Phenanthryl



9a Ar= 2-Naphthyl
9b Ar= 1-Naphthyl
9c Ar= 9-Phenanthryl



10a R^1= Me, R^2=H
10b R^1= H, R^2=Me



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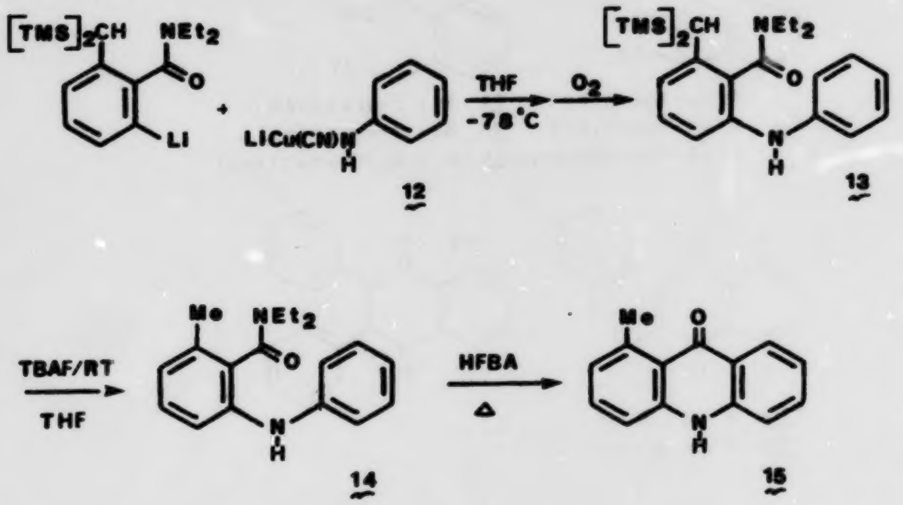
In light of recent work from our laboratory on the ortho N-aryl amination of tertiary benzamides[24], the above outlined silicon protection methodology will allow access to methyl substituted acridone systems and hence the corresponding AZA-PAHs.

In the exploratory example thus far investigated, the lithiated species of 2 **(Scheme 4)** was treated by oxidative coupling with the anilido-cyano cuprate 12 [25] to give the protected N-arylanthranilamide 13 in 48% yield. Subsequent desilylation using TBAF in THF afforded the methyl substituted N-arylanthranilamide **14** in 95% yield which was then cyclised to the corresponding 1-methyl acridone **15** in 50% yield[26] by refluxing in heptafluorobutyric acid for 36 hrs[24].

Scheme 4.



CONCLUSIONS

The tactic of silicon protection of ortho methyl functions has thus allowed direct and brief routes to peri-methyl substituted PAH precursors. Combined with the recently developed work in electrophilic ipso desilylation in systems of the type 1 [27], a method that has hitherto received limited application in synthesis[28], silicon protection in the directed metalation strategy may, in the future, provide access to a variety of polysubstituted PAHs. Work in this area is presently under investigation.

ACKNOWLEDGEMENTS

We thank the Ontario Ministry of the Environment (Air Resources Branch) for financial support.

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18. All new compounds show analytical and spectral data in agreement with the assigned structures.

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21. Readily prepared in 92% yield in a one-pot sequence as follows: 1. 1 equiv s-Buli/TMEDA/-78°C/45 min; 2. 1 equiv Me_3SiCl/-78°C/45 min; 3. 1 equiv. s-Buli/TMEDA/-78°C/45 min; 4. 2 equiv Me_3SiCl/-78°C → rt/8 h.

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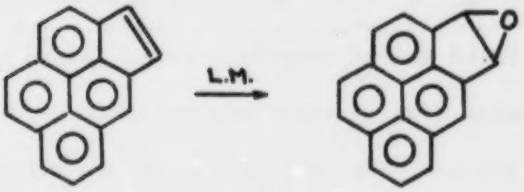
Synthesis of Polynuclear Aromatic Hydrocarbons of Interest in Environmental Pollution.

A Seminar for Presentation at Technology Transfer
Conference No. 4. Nov. 29-30, 1983

by E. Lee-Ruff.

The abundance and toxicity of certain polynuclear aromatic hydrocarbons (PAH) has been a matter of concern for a number of years[1]. The structural characterization of these in minute quantities as is available in environmental samples depend on the availability of well-defined reference standards. Furthermore the study of structure-activity patterns and understanding of their molecular action of toxicity require the availability of closely related model compounds. Recently certain PAH's having specifically a cyclopentene ring fusion have been found to be widespread environmental contaminants at the same time exhibiting potent mutagenic activity. One such derivative is cyclopenta(c,d)pyrene (1) which was recently characterized[2] and shown to give a positive test in the Ames assay[3]. The interest in these compounds arises from the unique structural features of 1 which has conspicuously absent the "bay region"[4]. Current understanding of the mode of action of these compounds in their toxic behaviour is that the PAH's are not the chemicals responsible but certain oxidized metabolites such as arene epoxides are susceptible to binding with nucleophilic sites in cellular macromolecules thus initiating the base-pair disruption mechanism and the onset of mutagenesis. The PAH's which exhibit such activity all have a common structural feature of having a "bay-region" as seen by the classical PAH benzo(a)pyrene 3 which is metabolized by liver microsomes to benzo(a)pyrene diol epoxide 4. These particular bay-region diol epoxides are prone to nucleophilic addition reaction with nucleophiles reacting at the epoxide function. In order to determine whether other structurally related PAH's (those having cyclopentene ring fusion and similar oxygen heterocycles) are present in environmental PAH samples we



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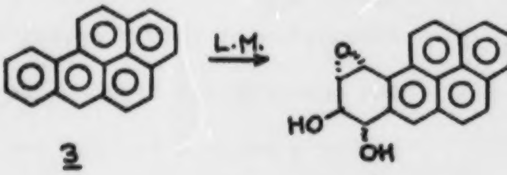
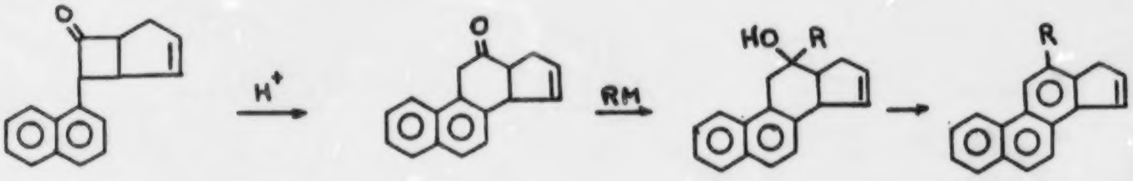


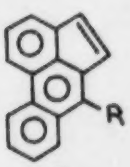
3 4

embarked on a program towards the synthesis of such derivatives. We had earlier developed a synthetic methodology for the preparation of polycyclic systems using cyclobutanones as intermediates^5. Such a method yield PAH's incorporating such rings as cyclopentene, furans and pyrans, an example of which is shown below:

Scheme 1



As is evident from this scheme it is possible to prepare specific alkylated PAH's by functionalizing the intermediate carbonyl containing compounds. Such compounds are of interest in that certain alkyl PAH's, specifically methyl



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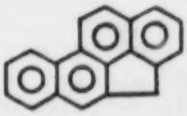

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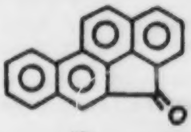

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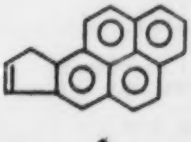

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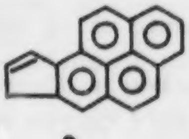

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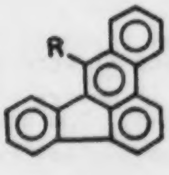

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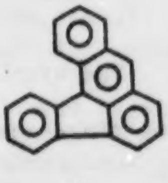

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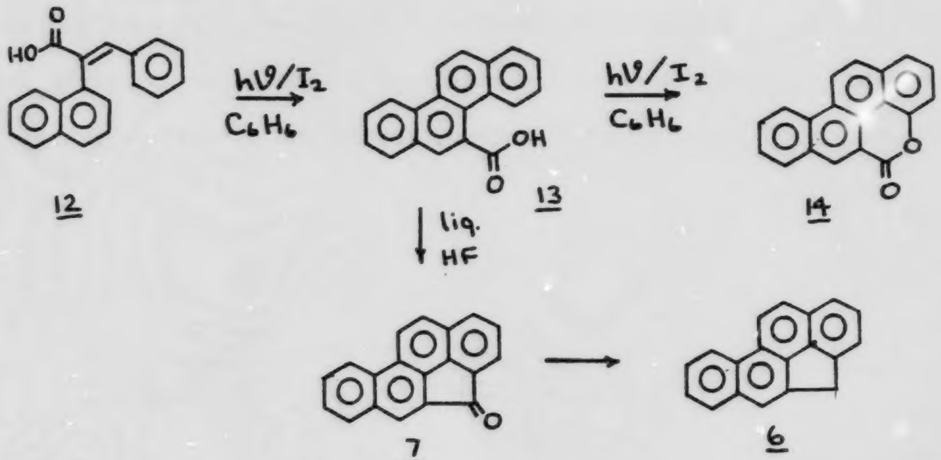
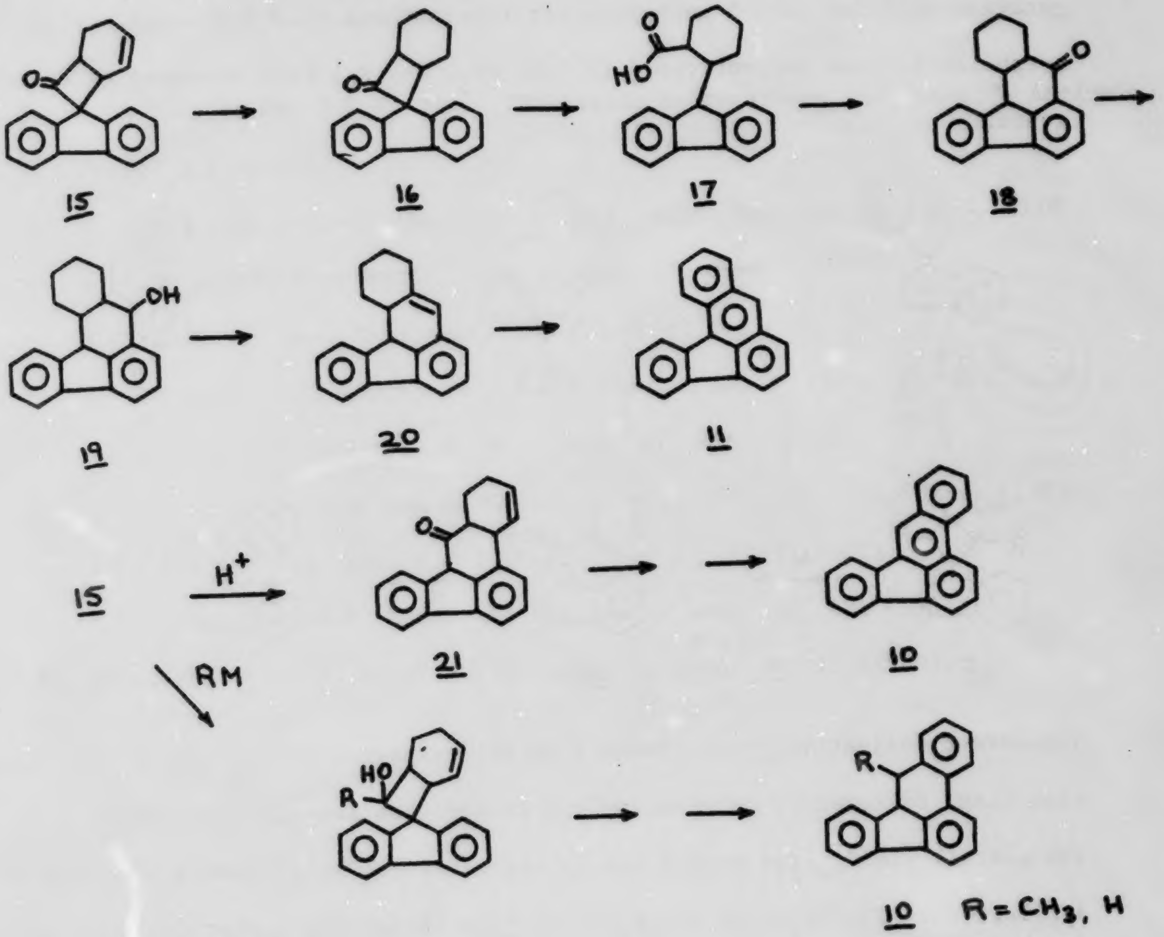
substituted PAH's, enhance the mutagenicity of these derivatives and are themselves found as environmental contaminants. The proposal was for the synthesis of the **above** PAH's to be used as reference standards in the identification of these in environmental samples. All of these compounds bear structural resemblance to either cyclopenta(c,d)pyrene and/or benzo(a)pyrene. Since our original submission of this proposal compounds 8 and 9 have been prepared by Harvey[6] and our original approach to 8 and 9 has been abandoned. We are reproducing Harvey's methods for the preparation of 8 and 9. We have recently accomplished the synthesis of 6 and 7 by the following scheme (2)[7]:

Scheme 2



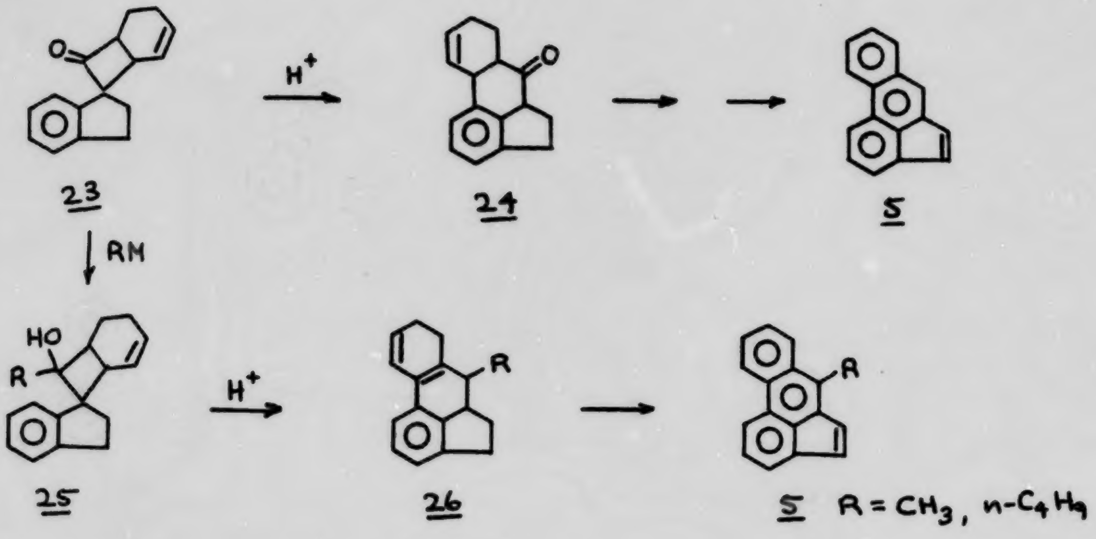
Photocyclization and oxidation of 12 gave chrysene-5-carboxylic acid and by-product 14 which was found to arise from secondary photolysis of 13. Lactone 14 has a π-electronic configuration identical with benzo(a)pyrene. Its toxicity and presence in environmental samples is currently under investigation. Cyclization of 13 to 7 was accomplished using liquid HF. The ketone (7) was reduced to 6 by Wolf-Kishner reduction. The overall conversion yield was 30% for 6. This particular synthesis is being reported in a forthcoming issue of the Journal of Organic Chemistry[7].

The synthesis of 10 and 11 were accomplished using our route of cyclobutanone ring-expansion reaction. The scheme involves a divergent pathway using the common cyclobutanone intermediate 15 which was obtained by cycloaddition of fluorenylideneketene and 1,3-cyclohexadiene. Hydrogenation of 15 gave 16

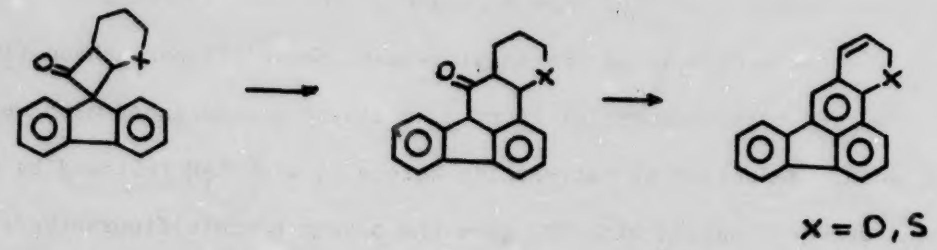


accompanied with over-reduced cyclobutanol. Ring opening of 16 or 15 with hydroxide proceeded efficiently to give 17 and its unsaturated analogue respectively. Hydrogenation of its unsaturated analogue produced 17 which could be readily cyclized and dehydrated to 18 with liquid HF. Reduction of 18 with LAH followed by acid dehydration and dehydrogenation with DDQ gave benzaceanthrylene 11 which showed identical physical and spectral data as reported recently for the same compound[8]. The same intermediate 15 could be used for the synthesis of the angular-fused benzo(b)fluoranthene (10). Acid-catalyzed rearrangement of 15 to 21 could be accomplished with methansulphonic acid. Reduction of tetracyclic ketone 21 with LAH followed by dehydration and dehydrogenation with DDQ gave the parent benzo(b)fluoranthene 10.

The methyl derivative 10 R = CH_3 could be prepared by way of methylation of 15 with $LiCH_3$ to alcohol 22. Acid-rearrangement of 22 followed by dehydrogenation with DDQ gave 5-methylbenzo(b)fluoranthene in 20% overall yield. The acephenanthrylene derivatives 5 (R = H, CH_3, n-C_4H_9) were prepared by a similar route.



The parent derivative 5 was prepared by acid-rearrangement of 23 to 24. Reduction (LAH) followed by dehydration and dehydrogenation gave the parent 5 in 25% overall yield. The methyl and n-butyl derivatives of 5 were prepared by alkylation of 23 (CH_3Li or n-C_4H_9Li), to 25, acid-catalyzed rearrangement to the tetrahydroderivative 26 followed by dehydrogenation to 5. Overall yields of 5 ranged from 20 to 30%. All of these compounds are currently being tested for their mutagenic activity[9] and presence in air-particulate samples. Further work in this area is being carried out to extend such syntheses in the preparation of oxygen and sulphur heterocycles as shown below.



An example of the above synthesis has been achieved for an oxygen heterocycle[10].

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RETROSPECTIVE CORRELATION SPECTROSCOPY AND ITS APPLICATIONS TO ATMOSPHERIC MONITORING

R.W.NICHOLLS, M.W.P.CANN AND K.DICK
Centre for Research in Experimental Space Science
York University
4700 Keele St., Downsview Ontario M3J 1P3

ABSTRACT

A brief review of given of the concepts of ANALOGUE correlation spectroscopy in the context of atmospheric monitoring. It was proposed and developed in a number of laboratories in the 1960's. A number of commerical field correlation instruments have been developed for remotely sensing the presence and concentration of atmospheric contaminant species. These instruments use a unique correlation mask for each of the species, and thus sense one species at a time.

We have made a study of the potential of the related method: RETROSPECTIVE digital correlation spectroscopy for the concurrent sensing of a number of atmospheric contaminants , for each of which a unique digital correlation mask is specified, and convolved numerically with the digital field spectrum.

The results of this "proof of concept" study is described and the aims of the recently started research programme to apply the concept to atmospheric spectra is reviewed.

1: INTRODUCTION

Analogue field correlation spectrometers are commonly used for the monitoring and diagnosis of air quality in the vicinity of contaminant emission targets near the observing site.

The concept of ANALOGUE CORRELATION SPECTROSCOPY was proposed and developed by a number of workers in the 1960's (Barringer, 1964; Barringer and Shock, 1966; Bottema, Plummer and Strong, 1964a,b; Kay, 1967; Williams and Kolitz, 1968; Millan, Townsend and Davies, 1968; Millan, 1972; Millan and Hoff 1977). It has been incorporated into the design of a number of commercial instruments, principally by Barringer Research Ltd. of Rexdale, Ont.

In the analogue instruments, the field spectrum is repetitively compared with the simulated laboratory spectrum of one of the suspected molecular contaminants. The laboratory spectrum of the contaminant is simulated in a mask of alternate transparent and opaque slits. The transparent slits are arranged in a pattern which reproduces at the focal surface of the spectrometer, the pattern of strong spectral absorption features of the contaminant of interest. Correlation between the field spectrum and the mask (from which quantitative estimates of the concentration of contaminant can be inferred) is achieved by relative oscillation, at the focal surface, between the mask and the field spectrum. This is followed by phase-sensitive photo-electric detection of the resulting signal passing through the mask.

If $S_1(L)$ and $S_2(L-x)$ are the respective intensity distributions of spectrum (1), and spectrum (2) offset in wavelength L by x, then the cross-correlation or co-variance between them is:

$$C(x) = \int S_1(L)\, S_2(L-x)\, dx \qquad (1)$$

When applied to the analogue correlation spectrometer, $S_1(L)$ can be identified with the field spectrum and $S_2(L-x)$ can be identified with the offset and oscillating correlation mask. After suitable calibration of instrumental response, the real-time readout of the time averaged C(x) can be interpreted in terms of number density or column density of the molecular atmospheric contaminant whose absorption spectrum is simulated by the correlation mask.

In the design and operation of these instruments, great attention has been paid to the optimization of the mask arrangement for each of the species (Millan and Hoff 1977). The analogue mask-correlation instruments are best suited to detection and monitoring of molecular species whose low resolution absorption spectra consist of a few unique well separated features, such as is trhe case for the SO_2 absorption bands between 300 and 310nm.

While the mechanical correlation mask can well represent low resolution spectra with separated and distinctive features, it is much less well suited to the representation of complex, multi-line, spectra, particularly at high resolution. Fabrication of individual correlation masks for such spectra poses almost insuperable mechanical difficulties. Another limitation of the analogue method is that a uniquely fabricated mask is needed for each species to be sensed, and only one species can thus be sensed at a time with one instrument.

2:THE RETROSPECTIVE DIGITAL CORRELATION METHOD-PAST WORK

The principle of the correlation method is sufficiently powerful that it appeared to be worthwhile to investigate methods by which it can be applied concurrently to complicated, and possibly high resolution spectra of important species. We were encouraged to try this approach from the clear identification of NO_2 bands in the optical depth spectra of urban Toronto haze, particularly under conditions of "Brown Haze", even though our optical depth spectra are at relatively low resoluiton. (Nicholls, Peterson and Bunn, 1981)

Our spectra are recorded digitally and maintained in a digital database for more than 3000. It has been steadily augmented since 1977.

We therefore propsed to NSERC, with the support of Barringer Research Ltd. that it would be very worthwhile to investigate the potential of **RETROSPECTIVE CORRELATION SPECTROSCOPY** for the quantitative remote sensing of atmospheric contaminant species. This suggestion was accepted and we were awarded a P(roject) R(esearch) for A(pplication) to I(ndustry) grant ot make a numerical assessment of this method.

The detailed results of that study were given in the final report (Nicholls, 1982) and in a paper attached as an appendix to the report.(Cann and Nicholls, 1984)

In the retrospective digital method it is assumed that the field optical depth spectrum $S_1(L)$ is recorded and available in digital form. Then in principle, a digital numerical correlation mask $S_2(L-x)$ can be developed for each species of interest using the methods of numerical spectral synthesis. We have developed extensive software for this and applied it to a wide range of spectral diagnoses in laboratory, atmospheric, cometary and astrophysical circumstances from the far ultraviolet to the microwave region of the spectrum. An example of such work applied to long path absorption ballon spectroscopy of the stratosphere is described in the paper by Cann et al(1979).

A digital mask can be synthesized from knowledge of molecular constants, from which line locations, profiles, and intensities can be calculated. It can also be produced by digitizing laboratory spectra of the species in question.

After some preliminary empirical studies on our field optical depth spectra, we decided to adopt model numerical field synthetic spectra and model numerical correlation masks. We investigated how the resulting correlograms depended upon the character of the model spectra, model masks, resolution, "noise" content of the spectra etc. Details of this work will be given in the oral presentation of this paper. The results confirmed that in principle there is no reason why field spectra cannot meaningfully be assessed for presence of more than one contaminant species at a time. Whether this is done retrospectively (by bringing digital records of field spectra back to the laboratory computer) or whether it is done in situ , using a dedicated computer system while recording field spectra will depend on circumstances. Both approaches are practical.

3: THE RETROSPECTIVE CORRELATION METHOD-FUTURE WORK

The results of this feasibility study were sufficiently encouraging that we proposed to the Ministry of the Environment an extension of the work oriented directly towards specific molecular species of importance in urban air quality assessment. The Ministry very recently approved the proposal and work has been in progress for a few weeks.

The proposed research programme has the following components:

1: Using the earlier study as a springboard, it is proposed to develop optimum digital correlation masks for spectral features of molecular species of importance to air quality considerations of urban and other atmospheres. Species such as NO, CO, NO_2, SO_2 are currently being studied.

2: Using methods of numerical spectral synthesis (Cann et al 1979), the long path atmospheric absorption signal (against daylight) will be realistically synthesized for a number of model atmospheres containing different column densities of molecular contaminants such as those listed above.

3: Retrospective correlation studies between the functions generated in (1) and (2) will be carried out to optimize spectral conditions (resolution, free spectral range, digital correlation masks etc.) for correlations with specific molecular species.

4: A programme of field observations will be proposed in the light of experience gained in tasks 1,2 and 3.

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Monitoring Genotoxicity in the Environment Using Sister Chromatid Exchanges in Mice *

Authors: M. L. Petras, B. Nayak and R. Piscitelli
Department of Biology,
University of Windsor,
Windsor, Ontario

To be presented at: Technology Transfer Conference No.4
Ministry of the Environment, Ontario
Constellation Hotel, Toronto

* Supported by a grant from the Ministry of the Environment of Ontario

ABSTRACT:

Preliminary evidence in a study by B.N. Nayak suggested that sister chromatid exchanges (SCE) in both wild and inbred mice might provide us with a sensitive system for monitoring changes in environmental levels of genotoxic agents. This report describes attempts to increase the efficiency of the SCE test and some early results in testing the sensitivity of the system.

The SCE test involves differential staining of newly formed chromatids through the incorporation of 5-bromo-2'-deoxyuridine (BudR). BudR was introduced into mice by four routes and in several concentrations. Of these 80 mg/g body weight, serially injected intraperitoneally gave the most consistent results in both C3H (5.40±0.14) and wild mice maintained in the laboratory (5.56±0.12); BudR even in very high doses in drinking water resulted in poor differential staining; plain BudR tablets gave consistently high SCE values (9.07±0.58); and parafin coated tablets gave values which were low and consistent in C3H (4.18±0.10) but which fluctuated considerably in wild mice because in some cases very little BudR was absorbed. For subsequent work, serial injections were used although coated tablets may be more suitable if the quality of the coating process is improved.

For a monitoring system to be useable, a large number of samples must be examined which in this case means that it must be feasible to prepare a large number of slides efficiently. This was accomplished by reducing the handling of slides. After the cells were fixed on a slide, the slides were placed in a staining tray. Then the entire tray was immersed in Hoechst 33258 for 15 minutes, exposed to florescent lights while covered with Sorensen's pH 8.0 buffer and stained with Fisher's Giemsa. The slides were not handled individually until they were examined under a microscope. Besides permitting the staining of a large number of slides this technique also gave excellent differential patterns.

During the development of the above procedure the effects of two mutagens, mitomycin C (MMC) and methyl methanesulfonate (MMS) injected intraperitoneally into the mice, were examined. The MMC increased SCE levels up to threefold at 2 ug/g body weight and the MMS fourfold at 10 ug/g body weight. For a more natural introduction of mutagens, MMS was placed in the drinking water for 14 days. SCE values doubled with a total intake as low as 23 ug/g body weight of MMS and tripled with 90 ug/g body weight.

Thirty-two wild mice were also examined. These were collected from corn cribs in southwestern Ontario either before or shortly after the area was sprayed with pesticides. The two sets of samples differed significantly in SCE counts, 5.99±0.32 for the "prespraying" sample and 9.22±0.63 for the "postspraying" group. The latter also differed significantly from the baseline SCE values observed in laboratory mice (5.40±0.14).

Results obtained thus far continue to support the potential of the SCE technique for mutagen monitoring.

(This study is supported by a grant from the Ministry of the Environment, Ontario.)

INTRODUCTION

Man through his efforts has either purposely or inadvertently released into his environs a plethora of agents harmful to his health and that of other organisms. Some of these agents are lethal, but many, especially at low concentrations, are much more insidious in their action. In some cases the latter may result in carcinomas years after the initial exposure, or in mutations which, if recessive, may not be recognized for several generations after they had occurred.(1-12) Needless to say there is a vital need for effective tests to monitor agents which cause genetic damage, that is genotoxins, in our environment. To date a number of tests have been developed to determine whether specific agents are mutagenic and/or carcinogenic.(13) Generally these tests have been routinely performed under controlled laboratory conditions and have been applied to specific carcinogenic and/or mutagenic agents.

This report presents some preliminary data on the feasibility of a general test for genotoxicity that would involve organisms exposed to possible genotoxins outside of the laboratory. That is a test that can be done using natural populations of animals and/or plants. Such a test would permit continuous monitoring of the environment by simply collecting samples from populations already in place and could provide us with an EARLY WARNING SYSTEM ON CHANGES IN GENOTOXIC LEVELS.

Among the tests which have been used to detect mutagenicity, there are four that appear to have the above potential. These include those which are involved in determining the incidence of chromosomal aberrations, dominant lethals, micronuclei and sister chromatid exchanges (SCEs). There are also several others which might be useful provided that sufficient genetic information is available on the organisms which are to be used. These include the detection of recessive mutations at specific loci and recessive lethals using inversion chromosomes. Unfortunately most of these are of limited value in studying genotoxicity in natural populations because either they are relatively insensitive or a lengthy and labourious breeding program is required. Only two appear to have much potential; these are the micronucleus test(14,15) and the sister chromatid exchange (SCE) test(16-19). Because Dr. B. Nayak (a former graduate student) and I had already been looking at chromosomal abberations in wild house mice (Mus musculus) for another purpose and because I and my students had been studying populations involving this species for a number of years, we selected the SCE test in this species for closer evaluation.

In deciding on the SCE test we were aware that many mutagenic and carcinogenic agents increased SCE levels and also that there were some exceptions.(16) Table 1 summarizes the results of a survey by Abe and Sasaki (20) regarding the correlation between SCE inducers and mutagenic/carcinogenic agents. They calculated the predictive value of the SCE test for both mutagenicity and carcinogenicity to be about 80%. We also knew that there has been considerable discussion on the nature of SCEs and on exactly what it is that the SCE test is measuring. Such discussion is irrelevent to our purposes as long as there is strong correlation between SCE levels and the doses of mutagenic/carcinogenic agents to which the organisms or tissues have been exposed.

In our approach we were concerned with:

1) modifying the technique used on laboratory mice to wild mice and then to determine an SCE baseline level for inbred mice maintained in our laboratory and to compare this to values obtained in freshly caught wild

mice;

2) possible genetic differences between inbred and wild mice which would effect SCE levels;

3) improving the consistency of the technique and making it less labour intensive so that more animals could be surveyed; and

4) determining the exposure levels that would increase SCE levels significantly.

APPROACH USED

Mice from three inbred strains, C3H/J, C57Bl/6J and DBA/2J were used to give us a baseline against which we considered all other animals. The wild mice came from corn cribs in southwestern Ontario. Corn crib populations were selected because large numbers of animals could be obtained when cribs were being emptied and because mice from different cribs would all have been exposed to the same basic diet and the same general environment. Heterogeneity in the environment related to farming practices and/or atmospheric fallout will of course occur.

To determine the effect of the environment in different parts of southwestern Ontario on inbred mice, C3H mice were housed in outdoor enclosures filled with corn. These were placed at various sites throughout the trapping area (Figure 1).

At the outset of this study, the mice were given intraperitoneally, 9 hourly injections of 40 ug/g 5-bromo-3'-deoxyuridine (BrdU) and 2 ug/g fluorodeoxyuridine. This was followed by 4 ug/g of colchicine 27 hours after the first BrdU injection. The mice were sacrificed 3 hours later. Femurs were removed and the bone marrow cells were flushed into warm (37C) 0.075M KCl. The cells then went through five stages of fixation. After the slides were made, they were aged for 48 hours, then heated to 87-89C for 15 minutes in a phosphate buffer and stained in 5% Fisher's Giemsa. This is basically the Korenberg and Freedlender (21) technique.

INITIAL RESULTS

SCE values observed in the inbred strains studied, in wild caught mice maintained in the laboratory for 6 months, in wild caught mice injected with BrdU within 24 hours of capture and in C3H housed in cornfilled enclosures for four weeks are summarized in Table 2.

These results show that the three inbred strains gave very similar SCE values and that the wild mice housed under laboratory conditions for an extended period gave SCE values that fall well within the range of the inbreds. Therefore the genetic differences which occur between the inbred and the wild did not seem to have a major effect on SCEs. Newly caught mice gave significantly higher SCE values than inbreds or laboratory maintained wild mice. Similarly, the inbred mice housed outdoors showed a significant increase in SCE levels over those housed in the laboratory.

Table 3 gives more detailed results of the wild mice collected from different sites in southwestern Ontario. An analysis of the mean SCE/cell/mouse/site revealed a negative correlation between the SCE level and the distance to the nearest major industrial site (r= -0.79). The centres considered were the Detroit-Windsor complex, Sarnia and Toledo, Ohio.

REVISED PROCEDURE

The procedure used to obtain the early results gave slides of varying quality. Sometimes for no apparent reason the chromosomes were either condensed and poorly spread or no differential staining was seen. Furthermore, the procedure was very time consuming, especially the fixing and staining stages.

In the revised procedure 80 ug/g of BrdU were given intraperitoneally. The fixation phase was reduced to 90 minutes from over three hours. The slides were stained with Hoechst 33258 for 15 minutes in a concentration of 10 ug/ml of distilled water. The slides were then placed in a staining jar containing Sorensen's buffer at a pH of 8.0 and the containers, covered with saran wrap, were exposed to fluorescent lights for approximately 23 hours. At the end of this period a single slide was removed and stained with Fisher's Giemsa for 8 minutes. If no differential staining was seen the rest of the slides were exposed to the fluorescent lights for another ten minutes and another test slide was stained. This was repeated until good differential staining was observed and then all the remaining slides are stained. (We are grateful to Dr. Alena Krepinsky of York University for suggesting this staining procedure).

The revised procedure involves less effort because once the slides are made they are not handled individually until examination under a microscope. This then permits the preparation of a large number of slides at one time, and the test slides insure that no run is lost because of poor differential staining.

RESULTS WITH THE REVISED STAINING PROCEDURE

Table 2 also summarizes the results obtained with 80 ug/g body weight of

BrdU injected intraperitoneally and the new staining procedure. The baseline count in C3H mice is higher then with 40 ug/g body weight BrdU, 5.63+0.14, as is the SCE count of wild mice maintained under laboratory conditions for an extended period (5.72+0.15). Freshly caught wild mice again gave values that were higher than that seen in laboratory mice. In this case the wild mice could be subdivided into two groups: PRE-PESTICIDE-SPRAYING SAMPLES and POST-PESTICIDE-SPRAYING SAMPLES. The former gave SCE values only slightly higher than the laboratory mice while the latter approached three times the baseline value (Table 4).

Table 5 summarizes the results obtained in mice which had been exposed for one week in the population enclosures. These mice unlike the earlier group (Table 2) were injected with 80 ug/g body weight of BrdU. Of note is the fact that mice after a only a single week of exposure showed very high SCE values at some sites, almost three times the value seen in the laboratory mice.

FURTHER ATTEMPTS TO IMPROVE THE TECHNIQUE

Serial injection of BrdU into mice is time consuming and tedious, so we considered several alternatives:

1) Addition of BrdU to drinking water for nine hours beginning at the 29th hour prior to sacrificing the animal. Since BrdU is light sensitive the mice were kept in total darkness during this period. Doses ranging from 40 to 160 ug/g body weight were given. No differential staining was observed.

2) Implantation of uncoated 50 mg BrdU tablets (obtained from Dr. J. Heddle, York University). The mice were anesthetized with Avertin

(tribromoethanol), an anesthetic which totally inactivates an animal for about 20 minutes. The tablets were inserted subcutaneously between the scapulae. The SCE counts (Table 6) were considerably higher than in mice injected with 80 ug/g body weight/hour of BrdU. These results are consistent with those reported in the literature (22,23).

3) Implantation of paraffin-coated 50 mg BrdU tablets (obtained from Dr. R. Tice, Brookhaven Laboratory). The SCE levels (Table 6) were considerably lower than those observed with the plain tablets and somewhat lower than the values obtained with 80 ug/g BrdU injections. These levels would be suitable except that the amount of BrdU absorbed differed somewhat from mouse to mouse and at this point we do not know how much variability in SCE counts this caused. Paraffin-coated tablets will be tested further.

INTRODUCTION OF MUTAGENS

Mutagens can be introduced into an organism by injection, the respiratory system, the skin and the digestive tract. The normal laboratory procedure is injection. Much of the work, especially studies to determine the mutagenicity of a compound, has involved this route of introduction. This, however, is not the way compounds are taken in under natural conditions. We, therefore, began to compare the effects of intraperitoneal injection vs. oral intake of two compounds, methyl methanesulfonate (MMS) and mitomycin C (MMC). The objectives included determining the effects of: the time of exposure relative to BrdU treatment, the length of exposure for maximizing SCE induction and minimizing serious physiologic effects and different concentrations of a compound on SCE induction.

The initial step was to inject these compounds at different times prior to and after the initial injection of BrdU. Table 7, based on four mice in each time period and for each compound, shows that for both of these compounds the SCE induction increased as they were introduced closer to the beginning of BrdU treatment. MMC continued to induce SCEs even after the injection of BudR, whereas for MMS the SCE level stabilized. Some preliminary studies also suggested that injections of MMS and MMC had little effect on SCE levels if given 48 hours before the initial injection of BrdU.

As a first step in introducing mutagens "naturally" into the mice we decided to explore intake via drinking water since the amount taken in is easily quantified. Some error is encountered but the error with food intake or skin absorption is as great if not greater. Table 8 gives the results of one of the experiments we have done showing the relationship between the uptake of MMS and SCE induction. The MMS in this case was given over a two week period. SCE levels increased with increase in MMS intake ($r = 0.90$).

The questions that we are trying to answer, for MMS and MMC, should be extended to other compounds, such as cyclophosphamide and 7,12 diethylbenzanthracene, and should also involve a comparative analysis.

SUMMARY OF RESULTS

1) The revised SCE procedure is giving good chromosomal preparations with excellent differential staining consistently.

2) The differences between SCE levels in laboratory maintained and either freshly caught wild mice or inbreds maintained under natural conditions indicate that the approach is sufficiently sensitive to detect changes in the environment.

A seven day exposure of C3H mice to a natural environment was found to be sufficient to alter the SCE count.

3) The differences between the laboratory maintained inbred mice and freshly caught mice are not primarily the result of genetic differences but rather variability in the environment to which these animals are exposed.

4) Preliminary evidence suggests that pesticides have an effect on SCE levels. This must of course be explored further.

5) There appears to be a correlation between SCE levels and distances to nearest large industrial complex.

6) Coated BrdU tablets may be a suitable replacement for serial injections but the coating of the BrdU tablets must be improved and further evaluation is required.

UPCOMING STUDIES

There are also several additional aspects which should be addressed in the near future:

1) Validation of the SCE results by using another procedure such as the MICRONUCLEUS TEST.

2) Examination of possible synergistic interactions between several mutagenic/carcinogenic agents characteristic of a particular environment.

3) Increasing the sensitivity of the SCE TEST for low concentrations of mutagenic agents perhaps through the use of DNA repair inhibitors such as hydroxyurea and aphidicolon. Some preliminary studies using these inhibitors in tissue culture cells have been reported.(24)

CONCLUSION

Results obtained thus far continue to support the proposal that the SISTER CHROMATID EXCHANGE TEST is an effective early warning monitor of environmental genotoxins.

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TABLE 1. Correlation between mutagenicity and/or carcinogenicity of environmental chemicals and the SCE test.

MUTAGENICITY	CARCINOGENICITY	NO. OF CHEMICALS	SCE POSITIVE	SCE NEGATIVE
+	+	75	69	6
+	-	14	8	6
+	?	21	16	5
-	+	14	8	16
-	?	43	9	34
?	+	9	3	6
?	?	179	124	55
TOTAL		379	246	133

Based on: Abe and Sasaki (1982) SCE as an index of mutagenisis and/or carcinogenisis. In Sister Chromatid Exchanges: 461-514. A.A. Sandberg, ed. Alan R. Liss, Inc.

TABLE 2. Mean SCE values in inbred and wild male mice maintained under various conditions and given either 40 ug/g body weight or 80 ug/g body weight of 5-bromo-3'-deoxyuridine (BrdU).

MICE	CONDITIONS UNDER WHICH THEY WERE MAINTAINED	40 ug/g/injection BrdU			80 ug/g/injection BrdU		
		NUMBER	MEAN SCE ± S.E.M.*		NUMBER	MEAN SCE ± S.E.M.*	
C3H/J	Laboratory	32	3.39	0.07	18	5.63	0.14
C57Bl/6J	Laboratory	14	3.68	0.09	10	5.79	0.13
DBA/2J	Laboratory	9	3.92	0.13			
Wild	Laboratory for at least six months	18	3.52	0.06	9	5.72	0.15
Wild	Corn cribs - test was begun within 24 hours of capture	49	6.02	0.16	22	7.49	0.36
C3H/J	Enclosures in Essex County	14	5.62	0.14	12	10.27	0.34
C3H/J	Enclosures in Sarnia area	23	5.04	0.16			

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[*] Standard error of the mean was calculated from the SCE values of individual mice.

TABLE 3: SCE values in wild mice collected from various locations is southwestern Ontario. Only samples with two or more mice are included.

LOCATION	NO. OF MICE	MEAN SCE/CELL ± S.E.M.*	KM FROM WINDSOR/DETROIT INDUSTRIAL COMPLEX
Fingal	2	5.37 ± 0.13**	154
Wardsville	4	5.36 ± 0.53	115
Ridgetown	12	5.58 ± 0.35	99
Tilbury	4	4.83 ± 0.19	58
Stoney Point	8	6.38 ± 0.13	48
Harrow	4	7.23 ± 0.28	28
Essex	5	6.95 ± 0.13	14
McGregor	8	6.27 ± 0.31	12

* Standard error of the mean for a locality was based on the SCE values of individual mice.

** The above mice were given 40 mg/g body weight of BrdU. The C3H control values were 3.39.

TABLE 4: SCE values observed in mice collected from corn cribs in southwestern Ontario before and after spraying was begun.

LOCALITY	DATE	NO. OF MICE	SCE/CELL	SEM/SAMPLE
PRESPRAYING:				
Trudell	4/26/83	4	5.84	0.66
Laramie	5/11/83	2	5.74	0.30
Sinasac	5/11/83	4	6.47	0.33
Rocheleau	5/26/83	2	5.60	1.61
Pooled:		12	5.99	0.32
POST SPRAYING:				
Houle	6/25/83	6	7.66	0.21
Glendening	7/5/83	4	11.55	1.56
Pooled		10	9.22	0.86

Prespraying and Postspraying results are significantly different (0.5% level).

TABLE 5: SCE values in C3H males placed in outdoor enclosures filled with corn and in appropriate controls maintained in the laboratory.

SITE	DISTANCE FROM AMHERSTBURG	ONE WEEK'S EXPOSURE
Delmore	3.8 km	10.50 ± 1.16
Paquette	5.8 km	7.30 ± 0.81
Laramie	8.1 km	9.35 ± 0.90
McKim	20.8 km	12.10 ± 1.23
Trudell	61.2 km	14.10 ± 1.76
Belanger	72.5 km	8.24 ± 1.03
Controls		5.89 ± 0.49

TABLE 6: A comparison of SCE counts in C3H and wild mice either injected with BrdU or implanted with BrdU tablets.

MICE	NO.	TREATMENT	SCE/CELL	S.E.M.
C3H males	12	80 ug/g/hr	5.40	0.14
C3H males	3	plain BrdU tablets* (50 mg)	9.06	0.58
C3H males	5	coated BrdU tablets** (50 mg)	4.18	0.10
Wild males (Rocheleau)	2	"	5.60	0.23

* Obtained from Dr. J. Heddle, York University.

** Obtained from Dr. R. Tice, Brookhaven Laboratory.

TABLE 7. SCE values in C3H mice that received either 2 ug/g body weight of mitomycin C or 10 ug/g of methyl methanesulfonate at various times before and after the first injection of BrdU. Both compounds were dissolved in Dulbecco's phosphate buffered saline. Four mice were used for each chemical and each time period.

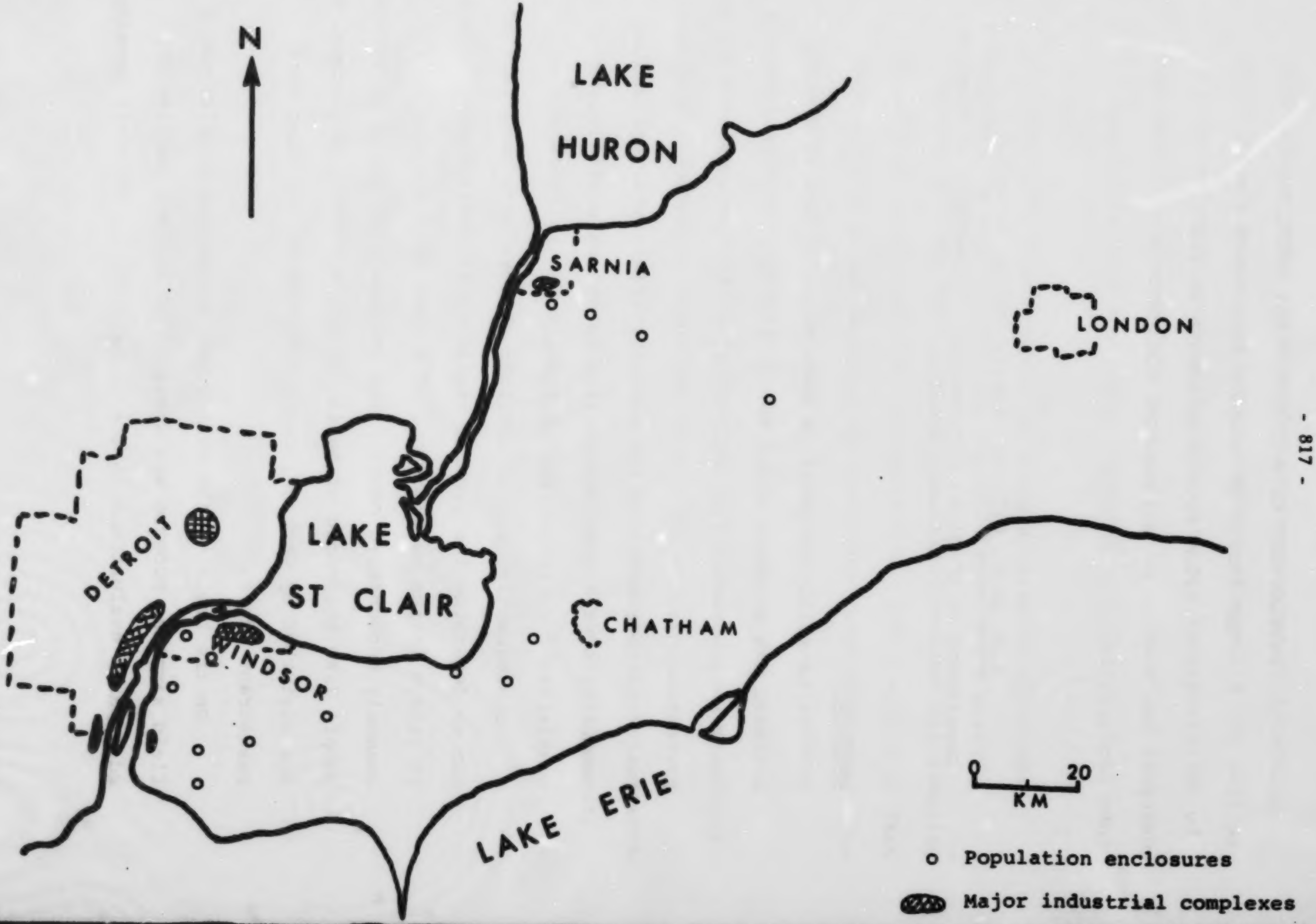
TIME (hours)	SISTER CHROMATID EXCHANGES	
	MITOMYCIN C	METHYL METHANESULFONATE
Control (no compound)	3.99	
-26*	5.88	7.67
-13	5.90	13.99
- 6.5		12.65
- 3.25		14.26
- 0.75	6.85	13.70
+ 2	7.75	
+ 6.5	8.61	
+13	11.88	14.16
+24	6.40	

* This refers to twenty-six hours before the initial BrdU treatment. All mice were injected with 40 ug/g body weight of BrdU.

TABLE 8: SCE levels and amounts of methyl methanesulfonate (MMS) taken in with drinking water by C3H mice. The SCE values are based on an average of three mice per concentration except for the last where only one mouse was available. The actual doses varied with water intake.

DOSE PLANNED (ug/g body weight)	AVE. WEIGHT OF MICE (g)	AVE. WEIGHT CHANGE (g)	TOTAL MMS INTAKE (mg)	SCE/CELL and SEM
0	31.1	+1.73	0	4.72 ± 0.02
2.5	25.4	+0.75	0.18	5.32 ± 0.23
10	28.4	+1.37	0.65	6.97 ± 0.85
40	28.6	+1.10	2.49	8.47 ± 2.68
160	26.6	+1.78	8.57	7.23 ± 0.06
640	28.4	-0.17	24.11	7.25 ± 0.24
2560	26.3	-4.80	72.01	12.25

FIGURE 1: Distribution of population enclosures in southwestern Ontario.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DEVELOPMENT OF A STRATEGY FOR PREDICTING THE IMPACT OF ODOROUS POLLUTANTS FROM FAST FOOD RESTAURANTS ON THE SURROUNDING COMMUNITY

by

A.W. Gnyp, C.C. St. Pierre and M. Poostchi

Department of Chemical Engineering, University of Windsor
Windsor, Ontario, N9B 3P4

ABSTRACT

It is still not possible to accurately assess whether citizens who complain about odors from fast food restaurants in their neighborhoods are truly offended by the emissions or are protesting against the litter and noise often created by patrons. Fast food operations do not normally affect the surrounding community over a wide enough area to generate sufficient odor complaints for statistical analyses.

Consequently, public attitude surveys have been carried out to determine the general reactions of individuals, selected at random in the neighborhood of a fast food outlet, to 25 commonly encountered odors. The results show that, in general, people like the odors of baking bread, barbeques and roses; but are disturbed by garbage, car/truck exhaust and fast food restaurant odors.

On the basis of the survey and documented complaints, a field sampling program was designed to collect samples for odor panel evaluations in the laboratory. A recently developed

Odor Impact Model, which will provide measures of detection, discrimination and complaint potential thresholds (as well as degrees of complaints), will facilitate the interpretation of laboratory generated data in terms of the number and frequency of complaints from the public reacting to a neighborhood odor source.

The Odor Impact Model is being applied to three odor sources. A domestic garbage landfill site and a sewage treatment plant provide facilities for convenient air sampling as well as good complaint documentation. The analysis of fast food outlets is handicapped by inadequate complaint data. The validated model can be used by industry and environmental regulatory agencies to characterize existing and proposed sources of odors with respect to their potentials for causing adverse community reactions.

A preliminary draft of a testing protocol for establishing the impact of an odorous source on the surrounding community is presented as a basis for future work.

INTRODUCTION

Of the various categories of air pollutants, odors are generally ranked as the major generators of public complaints to regulatory agencies in North American communities (1,2,3). Surveys of citizens living in the neighborhoods of odorous stationary sources indicate that odors can cause mental and physiological stresses on humans. Typical reactions are nausea, headache, loss of sleep, loss of appetite, impaired breathing and in some cases allergic reactions. However, objections against odors are generally raised, not because they are considered to be hazardous to human health, but because the human olfactory system is extremely sensitive to unpleasant stimuli (4).

Community odor complaints can be related to emissions from single or multiple fast food restaurants. Many of these convenience facilities appear to generate adverse reactions at considerable distances from their locations. In recent years these sources have received considerable attention from the public and regulatory agencies as more of them began to appear in the midst of residential areas and on the main streets of many North American cities (5). In spite of the alleged violations of air quality that communities have documented, it is still not possible to assess whether citizens who complain about odors from fast food restaurants in their neighborhoods are truly offended by the emissions or are protesting against the litter, traffic and noise normally associated with these operations. Although there are apparently widespread negative reactions to fast food restaurant odors, most communities do not generate sufficient complaints to regulatory agencies for statistical analyses.

Reviews of technical literature (6) and government documents (7) as well as discussions with personnel from the Ontario Ministry of the Environment (8) indicated that there was a pressing need for the development of a technological basis for odor control in North America before effective odor compliance programs could be implemented. It has been emphasized that the technological needs include:

- procedures for establishing the validity of spontaneous odor complaints
- development and testing of improved odor sensory measurement techniques
- critical evaluation of atmospheric dispersion models for prediction of ambient odor levels
- development of relationships between ambient odor levels and annoyance thresholds for different communities
- development of procedures for locating and defining the alleged source or sources causing an odor problem in a community.

In order to meet its obligations to the citizens of Ontario, the Air Resources Branch of the Ontario Ministry of the Environment provided financial support to the Air Quality Group in the Department of Chemical Engineering at the University of Windsor, through the Research Grants Programme. The objective and scope of this investigation were defined during several discussions involving members of the Air Quality Group and the Air Resources Branch (9,10,11). It was apparent that lack of objective measurements of source and ambient odor levels and community responses to odor episodes handicapped the abilities of North American regulatory agencies to deal with odorous stationary sources on more than a "nuisance" basis. Consequently, the Air Quality Group was expected to formulate guidelines which the Ontario Ministry of the Environment could use to develop codes regulating the sampling, emissions and controls associated with stationary odorous sources in general and fast food restaurants in particular.

In order to achieve this basic objective, a research programme was designed to facilitate:

- collection of representative odor samples from several fast food restaurants
- collection of ambient odor samples at various locations downwind from the facility at which source samples were being acquired
- correlation of ambient odor levels with source odor levels through refinements to the most appropriate atmospheric dispersion equation
- correlation of complaint potential thresholds and degrees of complaint of fast food restaurant odors determined under controlled laboratory conditions with citizen complaints actually registered with the Ontario Ministry of the Environment
- identification of any key odorant or odorants in the source emissions that could be responsible for community complaints.

PROGRAM DEVELOPMENT

A comprehensive examination of several fast food restaurants in the Windsor and Toronto areas showed that:

- there was a distinct lack of documented citizen complaints concerning fast food restaurants
- citizen responses could not be readily collected without creating unwarranted antagonism towards fast food restaurants in the community
- fast food restaurant emissions are too sporadic for routine ambient odor sample collection or real time instrumental monitoring of any key components.

In an effort to overcome these limitations a systematic search was conducted for odor sources where:

- citizen complaints to a regulatory agency are well documented
- emissions are relatively constant rather than sporadic
- emissions can be discussed in terms of readily identifiable major components
- existing odor control equipment operating parameters could be varied for assessment of odor removal efficiency and consequent changes in community responses.

During the past 4 years source and/or ambient odor sampling programs have been conducted at:

- an automotive foundry in Windsor, Ontario
- several fast food restaurants in Windsor and Toronto, Ontario
- a wastewater treatment facility in St. Catherines, Ontari
- a paint manufacturing facility in the Junction Triangle area in Toronto, Ontario
- a municipal solid waste landfill site in Avon Township, Michigan.

Automotive Foundry Studies

Preliminary evaluations of atmospheric dispersion models for predicting ambient odor levels were carried out during the summer of 1979 under the terms of an Ontario Ministry of the Environment Experience '79 grant (12). Ambient and source odor samples were collected simultaneously by 2 different teams. The source was an automotive foundry stack of 180 foot height with sampling ports installed at the 100 foot level. A newly developed in-stack dynamic dilution sampling head was used to dilute the stack gases with deodorized air to avoid condensation of water vapor and organic materials. Ambient samples were collected at 2 or 3 locations downwind of the stack. Since the foundry was emitting a dark, readily visible and odorous plume, it was easy to define the locations where the highest ambient odor levels were to be expected.

Fast Food Restaurant Investigations

Prior to any on-site sample acquisitions typical fast food restaurant odors were generated in the laboratory by subjecting hamburger patties, onions and french fries, supplied by a major fast food chain, to the cooking techniques pertinent to that organization. Examination of the resulting odors with a portable infrared gas analyzer suggested that oleic acid could be used as a key odorant for relating sensory odor levels to instrumentally measurable concentrations. This concept of a key odorant was used for laboratory generated as well as source samples in the development of an odor dose-response relationship that expresses odor levels (as key odorant concentrations) in terms of their real life dimensions which have a direct impact on a community as complaint potential thresholds and degrees of complaint (13).

The frequency and duration of odorous assaults on the surrounding community were determined with human observers and a portable infrared gas analyzer while source sampling of fast food restaurants was carried out in Windsor and Toronto.

Wastewater Treatment Facility Program

The impact of the wastewater treatment plant on the surrounding community was assessed in terms of total odor and hydrogen sulfide as a key odorant. Impingement levels were evaluated from measured source values through atmospheric dispersion modelling of steady-state emissions, using the traditional Gaussian plume dispersion relationship providing 1-hour averaging.

Paint Manufacturing Plant Studies

The source sampling program at a paint manufacturing establishment in the Junction Triangle area of Toronto indicated that a variety of technical and political problems were being created by the existence of multiple odor sources at a number of industrial sites. An intense community involvement attracted significant political attention.

Discussions with individuals familiar with the problems in this area showed that:

- public meetings were attended by Ontario Ministry of the Environment personnel as well as the local member of the Ontario Legislative Assembly
- area citizens as well as representatives of the Ministry of the Environment tracked offensive odors to sources at night and during daytime hours
- comprehensive ambient sampling/monitoring had been carried out by the Air Resources Branch mobile laboratory
- there was minimal or no documentation of the magnitudes of odor levels associated with any suspected source of odor emissions
- several industries had been issued with Ministerial Orders to improve their operations
- one industry had been charged with creating a neighborhood odor offence.

Solid Waste Landfill Site Studies

Validation of the Odor Impact Model can be a politically sensitive issue. Assessment of citizen reactions to any source odor insults must be carried out without generating an abnormal awareness of any odor problems that have existed in the surrounding community for some time.

A scarcity of reliable Canadian complaint data for Odor Impact Model validation fostered a cooperative program with the Air Pollution Control Division of the Michigan Department of Natural Resources, the Environmental Protection Division of the Department of Attorney General, State of Michigan and the Engineering Department of the Charter Township of Avon, Michigan. A 770 unit mobile home park, located less than 500 feet from a domestic solid waste landfill site serving 14 Michigan townships, provided a convenient study area. The Avon Township Engineering and/or Fire Departments registered citizen complaints and provided validation of complaints whenever personnel were available to visit complainants usually within 15 minutes after telephone calls were completed.

Experiences with the Community Survey used by the Air Pollution Control Division of the Michigan Department of Natural Resources indicated that the 6 questions were of a highly leading nature. They tended to encourage participants to respond towards the formulation of a predetermined conclusion. Consequently, a relatively neutral Public Attitude Survey was developed and modified through a series of pre-tests with individuals having various vested interests. The final version has been tested only once in the neighborhood of a fast food outlet. The completion of the 32 questions required less than 5 minutes.

RESULTS AND DISCUSSION

Automotive Foundry Studies

The Hemeon (14) simplification of the Gaussian plume dispersion equation, written in terms of source and ambient dilution ratios, underpredicted the ambient odorous impacts by factors of 100 to 200. Application of the Hogstrom (15) Fluctuating Plume Model, which recognizes that short term peak odor levels can be much higher than long term averages, provided even poorer correlation of predicted and measured impacts when detection thresholds were used as the basis of nuisance prediction.

Even if the Hemeon and Hogstrom models had provided better correlation of threshold data, it must be recognized that detection threshold data are only useful for estimating the atmospheric dispersion needed to make odorous source emissions non-detectable. Dravnieks and O'Neill (16) have suggested that in the neighborhood of a source where an odor is still perceived after atmospheric dilution, odor intensity may be a more important parameter than the odor detection threshold. Consequently, the concept of intensity was introduced into the Hogstrom Fluctuating Plume Model describing dispersion of short term puffs. Source and ambient odor intensities were evaluated according to the equal sensation function method using 250 ppm n-butanol as standard (12). Generally, the disagreement between experimental and predicted values of ambient odor intensity did not exceed a factor of 10.

Fast Food Restaurant Investigation

Figure 1 illustrates typical results of laboratory data defining an Odor Impact Model for fast food restaurant odors using oleic acid as the key odorous component. The Detection (fractional panel response) curve corresponds to panelists claiming to detect the presence of an odor because they actually smell something or because they are good guessors and can select the correct port delivering an odorous gas stream by chance. Since most panelists are not sure about the presence of an odor until they are exposed to much higher levels or concentrations, the concept of a Discrimination Threshold was introduced to account for the level at which panel members are absolutely sure that they can establish the presence of the odor under investigation. Depending on the quality (sour, burnt, fruity, acrid) and hedonics (pleasant or unpleasant) most people do not complain about an odor when they are first sure of its presence. The Complaint Profile or Annoyance Threshold has been defined as the level at which any fraction of odor panel members would be annoyed enough to complain when exposed to an odor for a specified period of time. The severity or degree of their complaints is measured in terms of the Annoyance Profile which is developed in an odor booth under controlled conditions using an arbitrary scale ranging from 0 to 10.

The relatively good agreement between the 50% panel responses for laboratory and source generated odors shown in Table 1 suggests that the use of oleic acid as a key odorant for fast food restaurant odors has considerable merit for future studies.

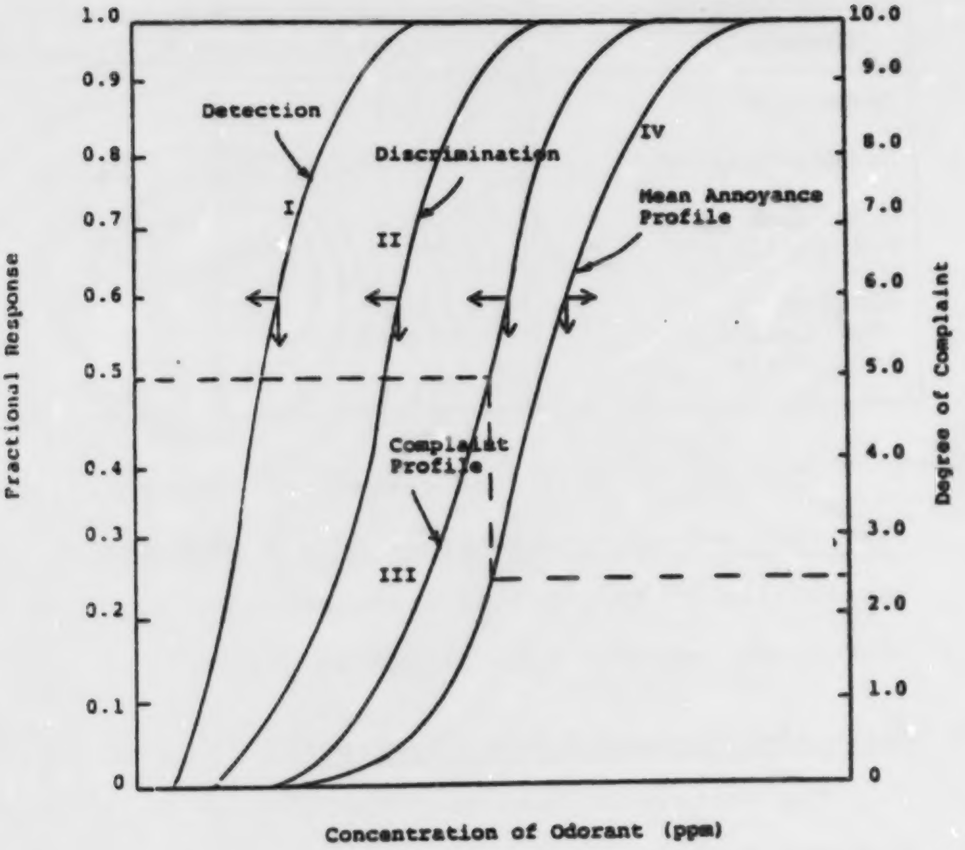

FIGURE 1: Illustration of Typical Odor Impact Model Profiles

TABLE 1: Comparison of Odor Impact Model Threshold Data (50% Panel Responses)

Odor Source	Laboratory Simulation	Fast Food Restaurant Exhaust	
Initial Oleic Acid Concentration	625 ppm	390 ppm	155 ppm
Threshold	Oleic Acid Concentration (ppm)		
Detection	3.9	5.5	3.9
Discrimination	7.7	11.2	7.8
Complaint Potential	8.4	11.3	7.8
(Degree of Complaint) 0-10 scale	1.6	2.2	1.8

Tracking of fast food restaurant odor emissions with human observers and a portable infrared gas analyzer defined the variability of impingement area locations and durations of odorous perceptions illustrated in Figure 2.

Wastewater Treatment Facility Program

The wastewater treatment facility at St. Catherines, Ontario provided a steady-state operation with essentially constant emission rates of hydrogen sulfide, total reduced sulfurs and sulfur dioxide which could be identified and collected at various locations as key odorants. The quality and hedonics of these odorous components created a well documented citizen complaint area which cou[illegible]e used to validate the results of atmospheric dispersion modelling.

Atmospheric dispersion of the steady-state emissions of

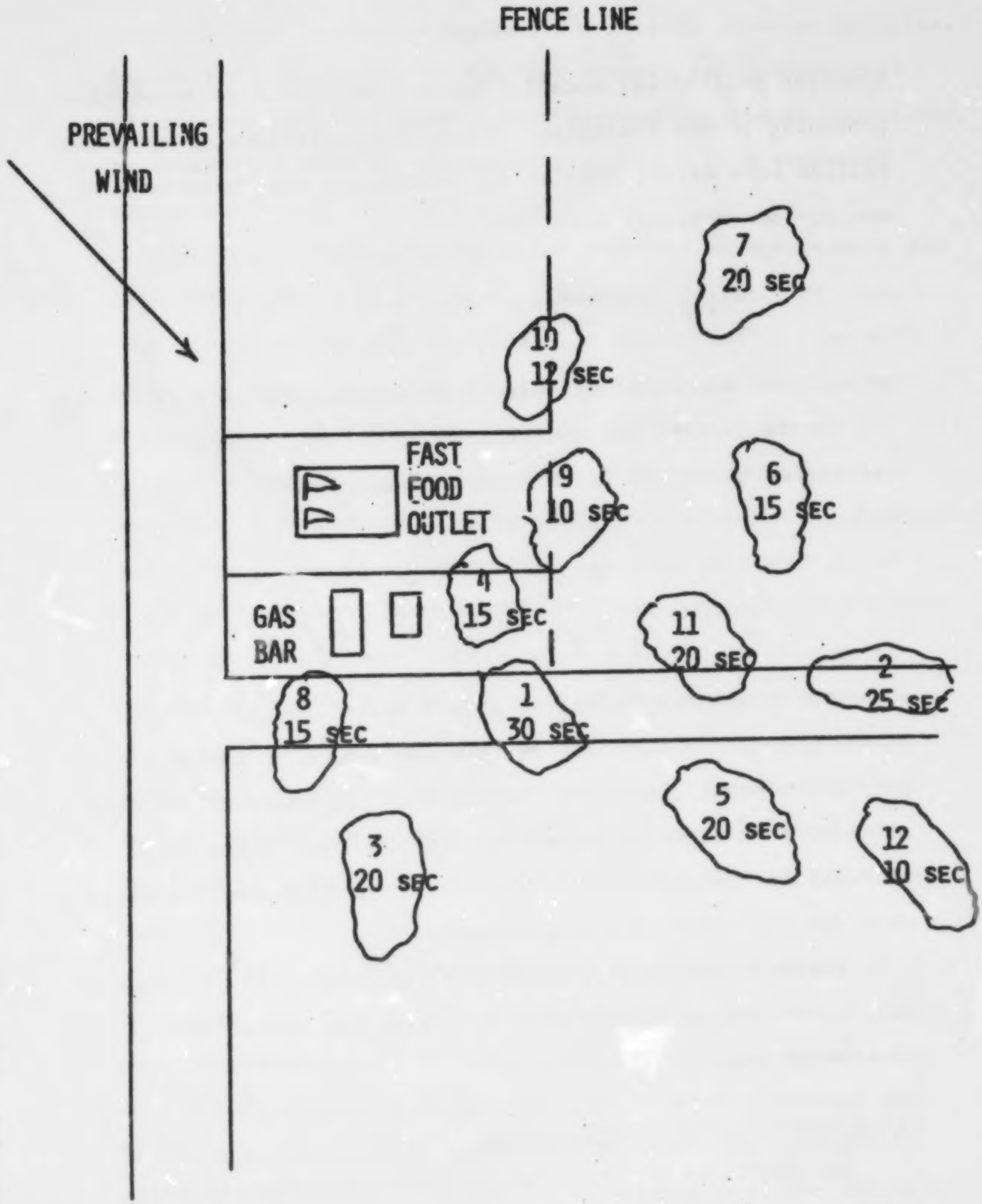
FENCE LINE
PREVAILING WIND
FAST FOOD OUTLET
GAS BAR
7 20 SEC
10 12 SEC
9 10 SEC
6 15 SEC
4 15 SEC
11 20 SEC
2 25 SEC
8 15 SEC
1 30 SEC
5 20 SEC
12 10 SEC
3 20 SEC

FIGURE 2: Typical Results of Tracking Fast Food Restaurant Odor Emissions

hydrogen sulfide was modelled using traditional relationships providing 1-hour averaging. Since concentrations of hydrogen sulfide were established at the source, it was convenient to use the conventional form:

$$C_{(x,y,o)} = \frac{Q}{\pi u \sigma_y \sigma_z} \exp\left[\frac{-y^2}{2\sigma_y^2} - \frac{H^2}{2\sigma_z^2} \right]$$

to estimate downwind impingement concentrations of this odorant.

To facilitate the modelling of total odor dispersion it was useful to modify the standard dispersion equation to the form:

$$N_{(x,y,o)} = \frac{N_s V_o}{\pi u \sigma_y \sigma_z} \exp\left[\frac{-y^2}{2\sigma_y^2} - \frac{H^2}{2\sigma_z^2} \right]$$

The dispersion modelling showed that impingement concentrations of hydrogen sulfide could be high enough to generate adverse community reactions at distances of 875 feet and 1560 feet from the exhaust stack for C and D atmospheric stabilities, respectively. Essentially similar results were obtained for total odor dispersion.

The majority of documented citizen complaints originated from homes located 900 to 1800 feet from the wastewater treatment plant.

Paint Manufacturing Plant Studies

The conflicts in the Junction Triangle community made it difficult for the Air Quality Group to be accepted as an objective organization trying to establish background information. A personal tour of the area indicated that significant odor problems were recognizable along the streets at sewer manholes. Apparently, discharges of scrubber liquors and perhaps even of products into the sewer system created unpleasant conditions in areas that were not necessarily downwind from suspected sources.

The paint manufacturing plant provided an opportunity for odor collection from 13 stacks. However, relation of source odor levels to citizen reactions and definition of complaint areas was hampered by the political climate in the community. Consequently, an arbitrary decision was made to define a "probable complaint area" in terms of ambient odor levels exceeding 1.0 odor units being sufficient evidence for "probable" complaint generation. Odor levels ranging between 0.5 and 1.0 odor units were assigned a rating of "possible odor impact". Predicted ambient odor levels below 0.5 odor units were projected to have minimal or zero effect on the surrounding community. Ambient impingement odor levels were calculated using appropriate modifications (in terms of source and ambient dilution ratios) of pertinent dispersion equations provided in Regulation 308 under the Environmental Protection Act (17).

Tables 2 and 3 illustrate the definition of areas of "probable" and "possible" odor complaints as determined by odor levels exceeding 1.0 odor units and ranging between 0.5 to 1.0 odor units, respectively. The 8 meter elevation odor levels are relevant for assessing the impact on neighboring 2-storey residences. It must be emphasized that the criteria used to define "probable" and "possible" complaint areas are

DISTANCE ABOVE GROUND 8.0000000 METERS

CONCENTRATION IN ODOUR UNITS

DISTANCE IN THE Y DIRECTION

DIST. FROM STACK	0	10	20	30	40	50	60	70	80	90	100
10.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
40.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
60.0	0.000	0.000	0.000	0.997	0.012	0.000	0.000	0.000	0.000	0.000	0.000
70.0	0.000	2.473	1.426	1.190	0.044	0.002	0.000	0.000	0.000	0.000	0.000
80.0	1.054	2.190	1.662	1.157	0.097	0.007	0.001	0.003	0.000	0.000	0.000
90.0	0.991	1.858	1.627	1.132	0.160	0.016	0.004	0.009	0.001	0.000	0.000
100.0	0.913	1.620	1.553	1.084	0.220	0.028	0.012	0.020	0.003	0.000	0.000
110.0	0.837	1.442	1.462	1.028	0.270	0.043	0.023	0.033	0.008	0.000	0.000
120.0	0.771	1.304	1.366	0.972	0.310	0.061	0.036	0.046	0.014	0.001	0.000
130.0	0.716	1.192	1.274	0.918	0.338	0.081	0.050	0.058	0.021	0.002	0.000
140.0	0.671	1.100	1.188	0.869	0.359	0.101	0.064	0.068	0.029	0.004	0.000
150.0	0.634	1.023	1.110	0.825	0.373	0.121	0.077	0.076	0.036	0.007	0.000
160.0	0.604	0.957	1.040	0.786	0.383	0.141	0.089	0.083	0.043	0.010	0.000
170.0	0.580	0.900	0.978	0.751	0.390	0.159	0.100	0.088	0.048	0.013	0.000
180.0	0.559	0.851	0.923	0.720	0.395	0.175	0.109	0.092	0.053	0.016	0.000
190.0	0.542	0.807	0.873	0.692	0.398	0.190	0.118	0.095	0.056	0.020	0.000
200.0	0.526	0.768	0.829	0.667	0.401	0.204	0.126	0.097	0.060	0.023	0.000
210.0	0.512	0.733	0.790	0.644	0.403	0.216	0.134	0.099	0.062	0.026	0.000
220.0	0.499	0.701	0.754	0.623	0.404	0.227	0.141	0.102	0.064	0.029	0.000
230.0	0.487	0.673	0.721	0.604	0.404	0.236	0.147	0.104	0.066	0.031	0.000
240.0	0.475	0.646	0.691	0.587	0.404	0.245	0.154	0.106	0.068	0.034	0.000
250.0	0.464	0.621	0.664	0.570	0.403	0.252	0.159	0.108	0.069	0.036	0.000
260.0	0.453	0.599	0.639	0.555	0.402	0.258	0.165	0.111	0.071	0.038	0.000
270.0	0.443	0.577	0.615	0.540	0.400	0.264	0.170	0.114	0.073	0.040	0.000
280.0	0.433	0.557	0.593	0.526	0.397	0.268	0.175	0.116	0.074	0.042	0.000
290.0	0.423	0.539	0.572	0.512	0.394	0.272	0.180	0.119	0.076	0.044	0.000
300.0	0.414	0.521	0.553	0.499	0.390	0.275	0.184	0.122	0.078	0.045	0.000
310.0	0.405	0.504	0.535	0.487	0.387	0.277	0.188	0.125	0.080	0.047	0.000
320.0	0.395	0.488	0.517	0.474	0.382	0.279	0.192	0.128	0.082	0.049	0.000
330.0	0.387	0.473	0.501	0.463	0.378	0.280	0.195	0.131	0.084	0.051	0.000
340.0	0.378	0.459	0.486	0.451	0.373	0.280	0.197	0.133	0.087	0.052	0.000
350.0	0.369	0.445	0.471	0.440	0.368	0.280	0.200	0.136	0.089	0.054	0.000
360.0	0.361	0.432	0.457	0.429	0.362	0.280	0.202	0.139	0.091	0.056	0.000
370.0	0.353	0.420	0.443	0.419	0.357	0.279	0.203	0.141	0.093	0.058	0.000
380.0	0.345	0.408	0.431	0.406	0.351	0.277	0.205	0.143	0.095	0.060	0.000
390.0	0.337	0.397	0.418	0.398	0.345	0.276	0.206	0.145	0.097	0.062	0.000
400.0	0.330	0.386	0.407	0.389	0.339	0.274	0.206	0.147	0.099	0.064	0.000
410.0	0.323	0.375	0.395	0.379	0.334	0.271	0.206	0.148	0.101	0.065	0.000
420.0	0.316	0.365	0.385	0.370	0.328	0.269	0.206	0.150	0.103	0.067	0.000
430.0	0.309	0.356	0.374	0.361	0.322	0.266	0.206	0.151	0.105	0.069	0.000
440.0	0.302	0.347	0.364	0.353	0.316	0.263	0.206	0.152	0.106	0.071	0.000
450.0	0.296	0.338	0.355	0.344	0.310	0.260	0.205	0.153	0.108	0.072	0.000
460.0	0.289	0.329	0.346	0.336	0.304	0.257	0.204	0.153	0.109	0.074	0.000
470.0	0.283	0.321	0.337	0.328	0.298	0.254	0.203	0.154	0.111	0.076	0.000
480.0	0.277	0.313	0.328	0.321	0.293	0.250	0.202	0.154	0.112	0.077	0.000
490.0	0.271	0.305	0.320	0.313	0.287	0.247	0.201	0.154	0.113	0.078	0.000
500.0	0.266	0.298	0.312	0.306	0.281	0.244	0.199	0.154	0.114	0.080	0.000

▣ Region of Probable Odour Complaints

☐ Possible Odour Complaints

TABLE 2: Odor Impingement Levels at an Eight Meter Height Resulting From All Sources

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DISTANCE   ABOVE   GROUND                                         0.0000000          METERS
                                  CONCENTRATION IN ODOR UNITS

                                      DISTANCE   IN   THE   Y   DIRECTION
DIST.
FROM
STACK     0       10      20      30      40      50      60      70      80      90      100
  10.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  20.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  30.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  40.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  50.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  60.0  0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  70.0  0.000   0.165   0.009   0.000   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  80.0  0.144   0.284   0.035   0.003   0.000   0.000   0.000   0.000   0.000   0.000   0.000
  90.0  0.230   0.394   0.086   0.016   0.001   0.000   0.000   0.000   0.000   0.000   0.000
 100.0  0.310   0.485   0.162   0.044   0.006   0.000   0.000   0.000   0.000   0.000   0.000
 110.0  0.375   0.554   0.253   0.088   0.016   0.001   0.000   0.000   0.000   0.000   0.000
 120.0  0.424   0.607   0.347   0.143   0.033   0.004   0.000   0.000   0.000   0.000   0.000
 130.0  0.458   0.646   0.434   0.205   0.056   0.008   0.002   0.001   0.000   0.000   0.000
 140.0  0.481   0.674   0.509   0.266   0.085   0.016   0.004   0.003   0.001   0.000   0.000
 150.0  0.496   0.693   0.569   0.324   0.117   0.026   0.007   0.005   0.002   0.000   0.000
 160.0  0.506   0.704   0.615   0.375   0.150   0.040   0.012   0.008   0.004   0.001   0.000
 170.0  0.511   0.709   0.649   0.418   0.183   0.056   0.019   0.012   0.006   0.002   0.000
 180.0  0.514   0.710   0.671   0.453   0.215   0.074   0.028   0.017   0.009   0.003   0.000
 190.0  0.514   0.705   0.684   0.482   0.244   0.093   0.038   0.023   0.013   0.004   0.000
 200.0  0.512   0.698   0.689   0.503   0.270   0.113   0.049   0.029   0.017   0.006   0.000
 210.0  0.509   0.688   0.689   0.518   0.294   0.133   0.061   0.036   0.021   0.009   0.000
 220.0  0.504   0.676   0.684   0.529   0.314   0.152   0.073   0.043   0.025   0.011   0.000
 230.0  0.499   0.662   0.675   0.535   0.331   0.170   0.086   0.050   0.030   0.014   0.000
 240.0  0.493   0.647   0.664   0.537   0.346   0.187   0.098   0.058   0.034   0.016   0.000
 250.0  0.486   0.631   0.651   0.537   0.358   0.203   0.111   0.065   0.038   0.019   0.000
 260.0  0.478   0.615   0.637   0.534   0.367   0.217   0.122   0.072   0.043   0.022   0.000
 270.0  0.470   0.599   0.622   0.530   0.375   0.230   0.134   0.080   0.047   0.025   0.000
 280.0  0.461   0.583   0.607   0.523   0.380   0.241   0.144   0.087   0.052   0.028   0.000
 290.0  0.453   0.567   0.591   0.516   0.384   0.251   0.154   0.094   0.056   0.031   0.000
 300.0  0.444   0.551   0.575   0.508   0.386   0.259   0.163   0.100   0.061   0.034   0.000
 310.0  0.434   0.535   0.559   0.499   0.386   0.266   0.171   0.107   0.065   0.037   0.000
 320.0  0.425   0.520   0.544   0.490   0.386   0.272   0.178   0.113   0.069   0.040   0.000
 330.0  0.416   0.505   0.528   0.481   0.384   0.276   0.184   0.118   0.073   0.043   0.000
 340.0  0.407   0.490   0.513   0.471   0.382   0.280   0.190   0.123   0.077   0.045   0.000
 350.0  0.398   0.476   0.499   0.461   0.379   0.282   0.195   0.128   0.081   0.048   0.000
 360.0  0.389   0.462   0.485   0.451   0.375   0.284   0.199   0.133   0.085   0.051   0.000
 370.0  0.380   0.449   0.471   0.440   0.371   0.284   0.203   0.137   0.088   0.054   0.000
 380.0  0.371   0.437   0.458   0.430   0.366   0.284   0.206   0.141   0.091   0.056   0.000
 390.0  0.363   0.424   0.445   0.421   0.361   0.284   0.208   0.144   0.095   0.059   0.000
 400.0  0.355   0.413   0.433   0.411   0.355   0.283   0.210   0.147   0.098   0.062   0.000
 410.0  0.346   0.401   0.421   0.401   0.350   0.281   0.211   0.150   0.100   0.064   0.000
 420.0  0.338   0.390   0.409   0.392   0.344   0.280   0.212   0.152   0.103   0.066   0.000
 430.0  0.331   0.380   0.398   0.382   0.338   0.277   0.213   0.154   0.106   0.069   0.000
 440.0  0.323   0.370   0.387   0.373   0.332   0.275   0.213   0.156   0.108   0.071   0.000
 450.0  0.316   0.360   0.377   0.364   0.326   0.272   0.213   0.157   0.110   0.073   0.000
 460.0  0.309   0.351   0.367   0.356   0.320   0.269   0.212   0.158   0.112   0.075   0.000
 470.0  0.302   0.341   0.357   0.347   0.314   0.266   0.211   0.159   0.113   0.077   0.000
 480.0  0.295   0.333   0.348   0.339   0.308   0.262   0.210   0.160   0.115   0.079   0.000
 490.0  0.289   0.324   0.339   0.331   0.302   0.259   0.209   0.160   0.116   0.080   0.000
 500.0  0.282   0.316   0.331   0.323   0.296   0.255   0.208   0.160   0.117   0.082   0.000
```

☐ Region of Possible Odour Complaints

TABLE 3: Ground Level Odor Impingement Resulting from all Sources

considerably more restrictive than those adopted by various jurisdictions for odor control purposes. Normally, residential zone odor levels are expected to be maintained below 2.0 odor units through regulations or criteria/objective standards (7,18).

It would be of considerable practical importance to relate the results of the Air Resources Branch mobile laboratory sampling/monitoring program to the "probable" and "possible" complaint areas established during this investigation.

Solid Waste Landfill Site Studies

The location of the 770 unit mobile home park with respect to the active landfill site is illustrated in Figure 3. Source odor samples were collected:

- during dumping of freshly collected garbage
- during dumping of incinerated garbage
- 18 inches above ground level where odors were detected
- above a stagnant pool of water through which gases percolated
- from a 2-inch observation well pipe sunk 30 feet below the cap of a completed cell.

Acquisition of representative odor samples during the dumping of garbage trucks or during sporadic gas evolution through the cap on a completed cell still represents a technical challenge. Reliable samples were collected above the stagnant water pool and from the observation well for the development of Odor Impact Models. Table 4 summarizes the Odor Impact Model threshold data for the 2 representative landfill samples.

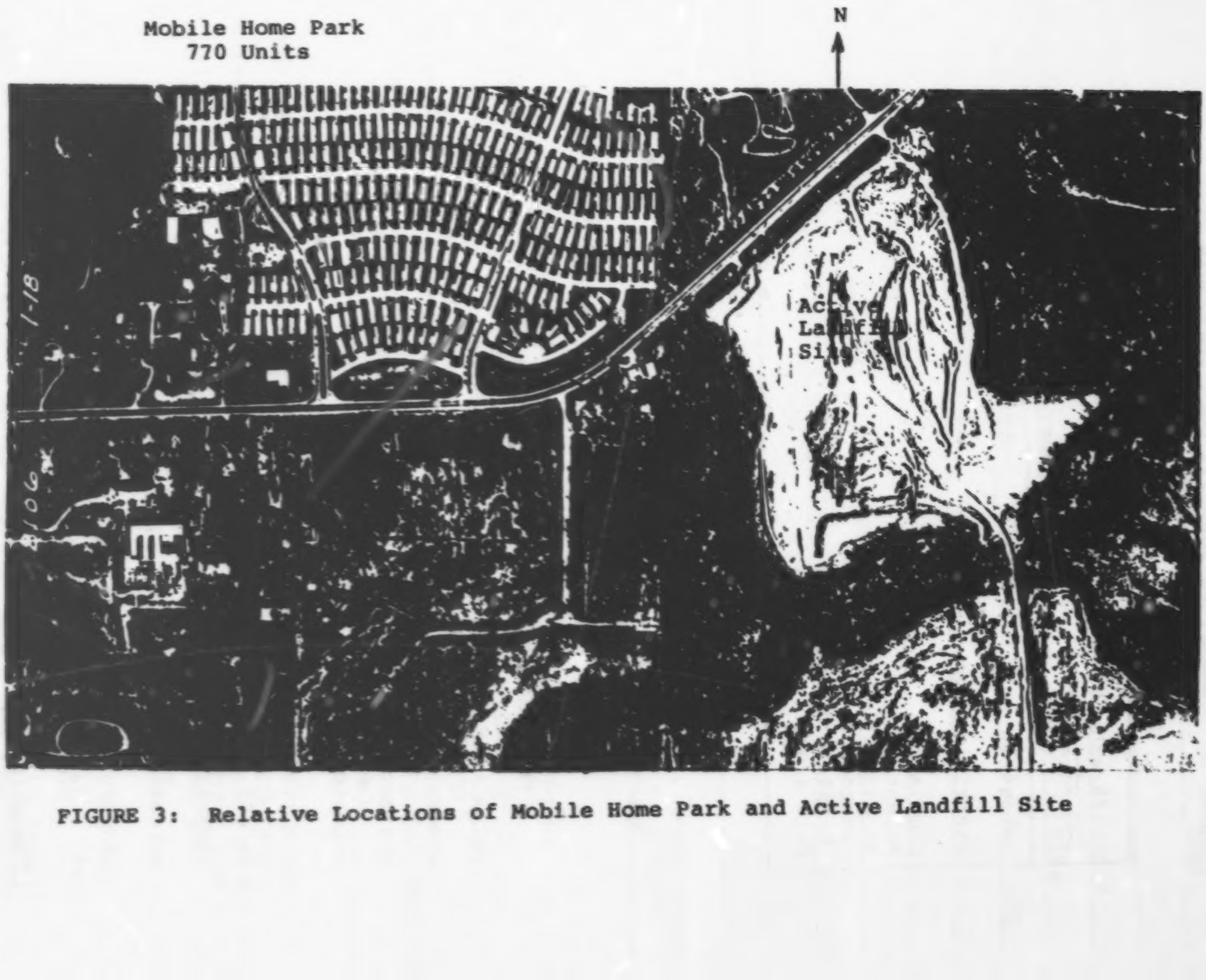
Mobile Home Park
770 Units
N
Active
Landfill
Site
1-18
106

FIGURE 3: Relative Locations of Mobile Home Park and Active Landfill Site

TABLE 4: Summary of Landfill Odor Threshold Data (50% Panel Responses)

Threshold (as odor units)	Odor Source	
	Water Pool	Observation Well
Detection	33	10,300
Discrimination	23	2,000
Complaint Potential	16	2,000
(Degree of Complaint) 0-10 scale	2.1	2.9

Tracking of odorous puffs across the top of the completed cells with 6 human observers indicated that under certain atmospheric conditions these puffs could move over considerable distances without losing their ability to create adverse receptor reactions.

Complaints from the mobile home park were most numerous during calm, humid evening or night time periods when apparent inversion conditions prevailed. Light winds from the southerly and easterly directions also generated 6 to 15 community reactions per day.

Figure 4 illustrates the Complaint Form used by the Township of Avon, Michigan to record citizen responses to the odors apparently transported from the landfill site. According

Std Distribution
Sed 10/19/83

AVON TOWNSHIP CITIZEN COMPLAINT REPORT

COMPLAINANT Philipson SEC# ______

ADDRESS 140 Fontainbleau

DATE 10-18-83 TIME 1825 A/P PHONE 652-3826

REPORT TAKEN BY: 107 DEPT. Fire

NECESSARY TO CHECK: IMMED: ___ WITHIN 24 HRS. ___ A.S.A.P. XXX

COMPLAINT Odor coming from the dump

1852 hrs. 105 advised odor was detected at time of investigation SEOCIA advised

ROUTING VERIFICATION BY: Bellhoff — Fire
NAME - DEPARTMENT

REPORT ROUTED TO:

___ DEPARTMENT OF PUBLIC SERVICE
___ ENGINEERING DEPARTMENT
___ BUILDING DEPARTMENT
___ HOUSING & ZONING INSPECTOR

ATTENTION: E. BORDEN

___ ASSESSING DEPARTMENT
X SUPERVISOR'S OFFICE
___ FIRE DEPARTMENT
___ OAKLAND COUNTY ROAD COMMISSION
___ OTHER (specify) ______

+ +

I HAVE ALWAYS HEARD THAT FOR EVERY CALL WE GET OF A COMPLAINT THERE ARE AT LEAST 25 or 50 OTHERS WHO ARE MAKING STATEMENTS LIKE "WHY DON'T THEY DO SOMETHING ABOUT THAT" BUT DON'T BOTHER TO CALL. I LIKE TO FEEL WE'RE HELPING MANY MORE PEOPLE THAN THE COMPLAINANT BY ADDRESSING THE CONCERNS BROUGHT TO OUR ATTENTION. E. Borden

INSTRUCTIONS FOR USE:

1) The person receiving the complaint fills out the top portion -who, what, when, where, and signs it after "Report Taken By".

2) The form (both white and cream parts still intact) is then forwarded to the Department Head of the complaint recorder. The Department Head checks the routing, signs after "Routing Verification By".

3) The white part is then sent to the Supervisor's office and the cream (larger) part sent to the action department.

FIGURE 4: Avon Township Complaint Form

to the data presented in Figure 5, some residents in the south east corner of the mobile home park have registered as many as 40 complaints in one year. Although more than half of all complaints have been validated through independent investigations by members of the Engineering or Fire Departments, frequent complainers are viewed with suspicion by regulatory agencies and the courts. In order to get a more objective assessment of the community odor problem, the Air Pollution Control Division of the Michigan Department of Natural Resources interviewed 42 residents in the neighborhoods surrounding the landfill site. The results of this community survey are summarized in Figure 6.

The format of the Michigan Department of Natural Resources Community Survey has been criticized because no efforts are made to control bias. As a result, the Air Quality Group developed the 32 question form described as Figure 7. A series of pre-tests was used for modification of the questions to insure that the questionnaire de-emphasized interests in any specific odor.

On a scale where +10 indicated liking an odor very much and -10 indicated liking an odor not at all, the odors associated with baking bread, roses and outdoor barbeques each scored a value of 6.0. On the other hand, the odors of ammonia, car/truck fumes and garbage received -8.2, -9.1 and -9.6 average ratings respectively. The odors characterizing Chinese restaurants, fried chicken and hamburger establishments were assigned respective magnitudes of -0.7, -1.3 and -2.2.

The responses to Question 32 are important in terms of

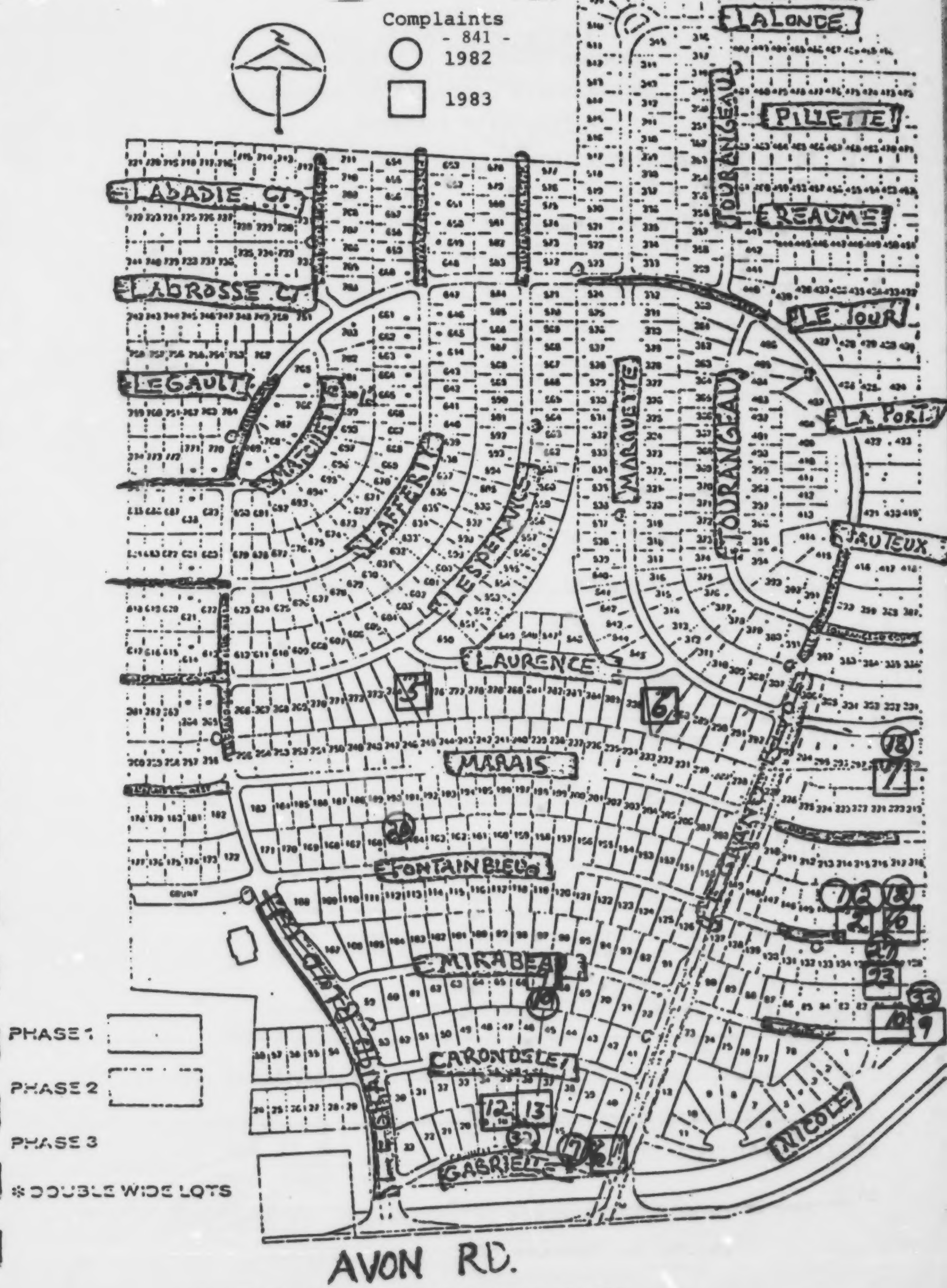
Complaints
- 841 -
1982
1983
LALONDE
PILLETTE
REAUME
TOURANGEAU
LE TOUR
LA PORT
JAUTEUX
LABADIE CT
LABROSSE CT
LEGAULT
MARCHETTE
LAFFERTY
LESPERANCE
MARQUETTE
LAURENCE
MARAIS
FONTAINBLEU
MIRABEAU
CARONDELET
GABRIELLE
NICOLE
PHASE 1
PHASE 2
PHASE 3
* DOUBLE WIDE LOTS
AVON RD.

FIGURE 5: Major Complaint Locations

AIR POLLUTION CONTROL DIVISION
MICHIGAN DEPARTMENT OF NATURAL RESOURCES
COMMUNITY SURVEY

42 Residents Surveyed

1. DO YOU NOTICE ANY POLLUTION PROBLEMS AROUND YOUR NEIGHBORHOOD?
yes 37 no 5.

2. IF SO, COULD YOU PLEASE DESCRIBE THE TYPE OF POLLUTION?
air 37 water - noise - aesthetic - other -

description: Stench; Musty; Rotten Garbage; Landfill smell; Smells Like Someone Died.

3. CAN YOU IDENTIFY THE SOURCE OF THE POLLUTION? yes 37 no 5

identification: Landfill site.

4. DOES THE POLLUTION APPEAR TO BE BOTHERSOME AT ANY PARTICULAR TIMES?

description: Late at Night; When winds from South; In Evenings when Real Humid; Depends on Wind Direction

5. HOW DOES THE POLLUTION AFFECT YOU OR YOUR PROPERTY? Makes Stomach Turn; Cannot Relax Outside in the Evenings; Bothers Friends who Visit; Visitors Leave; Could Not Sell Home; Affects Property Values; Some Nights Must Close Windows.

6. WOULD YOU DESCRIBE THE IMPACT OF THE POLLUTION ON YOU OR YOUR PROPERTY?
slight 6 moderate 11 very much 12 extreme 8 none -

description: God Help Us; Real Nuisance; Mostly Early AM: Bad Between Midnight and Noon; When Wind from Southeast.

NAME
ADDRESS
CITY
NUMBER OF RESIDENTS IN THE LIVING UNIT:
DATE: INTERVIEWED BY

FIGURE 6: Summary of Community Survey

University of Windsor

DEPARTMENT OF CHEMICAL ENGINEERING

Environmental Study

1. Age __________

2. Sex (circle one) M F

3. Address ______________________________
____

4. Occupation ____________________

5. Health (check one)
   - Excellent ☐
   - Good ☐
   - Average ☐
   - Fair ☐
   - Poor ☐

If problems, please list: ______________________________
____

The following statements relate to various odours normally present in the air. Please read carefully and then rate each one individually based on your personal reactions. To complete the question circle the arrow (↑) which most closely matches how you feel about it.

Example: I like baseball.

↑ ↑ ↑ [✓ ↑] ↑

Not at all O.K. Very much

6. I like the smell of cut grass.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

7. I like the smell of car and truck fumes.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

FIGURE 7: University of Windsor Environmental Survey

8. I like the smell of fried chicken outlets.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

9. I like the smell from the sewers.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

10. I like the smell of vinegar.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

11. I like the smell of garbage.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

12. I like the smell of a hospital.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

13. I like the smell at a Fruit Market.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

14. I like the smell of gasoline.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

15. I like the smell of hamburger restaurants in the neighbourhood.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

16. I like the smell of fresh popcorn.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

17. I like the smell of ammonia.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

FIGURE 7: University of Windsor Environmental Survey (continued)

18. I like the smell of paint.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

19. I like the smell of roses.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

20. I like the smell of beer.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

21. I like the smell outside a Chinese restaurant.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

22. I like the smell of a locker/dressing room.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

23. I like the smell of baking bread.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

24. I like the smell of a wood fire.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

25. I like the smell of chocolate.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

26. I like the smell of cigarettes.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

27. I like the smell of a carnival.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

FIGURE 7: University of Windsor Environmental Survey (continued)

28. I like the smell of peanuts.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

29. I like the smell of a barbeque.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

30. I like the smell of a leather jacket.

↑ ↑ ↑ ↑ ↑

Not at all O.K. Very much

31. What smells in the air seriously bother you? Please list:

____

____

____

____

____

32. This last question requires that you rank (in order of preference) 5 odors. Write the number which indicates your preference, below each odour.

1 = I like it best

5 = I like it the least

Example:	football	baseball	hockey	soccer	basketball
	③	②	①	④	⑤

Please rank:

Hay Swimming Pool Barbeque Hamburger Joint Garbage

Many thanks for your cooperation.

FIGURE 7: University of Windsor Environmental Survey (continued)

potential control of fast food restaurant odors with hypochlorite scrubbing solutions. Improper operation of proposed wet scrubbers could lead to more serious odor problems if the original fast food odors are replaced with the smell of chlorine. Preliminary results showed that the chlorine odor of a swimming pool is less acceptable than the odors associated with hamburger outlets.

CONCLUSIONS AND RECOMMENDATIONS

The ultimate objective of this investigation was to initiate the development of a scientifically and legally acceptable procedure for assessing the impact of odorous pollutants from stationary sources on the surrounding community. On the basis of studies at 5 different types of stationary sources, it is clearly evident that several technical deficiencies still exist with respect to the development of odor control regulations in North America.

Before the use of syringes and scentometers can be eliminated completely, basic criteria must be established for dynamic olfactometers that are to be used for evaluating source and ambient odor levels.

Uncertainties in odor detection thresholds can be minimized through application of probability models that account for the possibility of correct responses from panelists without true discrimination (19,20).

It is clearly evident that the application of atmospheric dispersion models to the prediction of ambient odor levels is still highly speculative. The Hogstrom Puff Dispersion

Model (15) provides a fundamental starting point in terms of dilution to threshold values or even more realistically in terms of odor intensities.

The early attempts by Dravnieks and O'Neill (16) and Lindvall and Radford (21) to relate ambient odor levels to annoyance thresholds require extension through the validation of the recently developed Odor Impact Model (13).

Recent experiences with legal problems involving odorous stationary sources (4,22) indicate that in order to verify that a suspected source (or sources) is or is not responsible for alleged odor problems in a community, it is necessary to prove that a scientifically and legally valid protocol has been followed with respect to data acquisition.

The protocol proposed on the basis of this investigation specifies measurements to be made at three levels involving the:

- source(s)
- ambient air
- affected population.

On-Site Measurements

In order to characterize the nature of any source emissions experimental programs will:

- identify the different locations where odors are released
- determine the frequency and duration of odorous emissions at each location
- determine the quantities (volumes) of typical odorous releases at each location
- establish the odor levels in typical releases at each location in terms of dilution to detection

thresholds, intensities or concentrations of key odorants

- identify, whenever possible, any key odorants that could be responsible for community complaints through infrared spectrometry and/or gas chromatography/mass spectrometry.

Off-Site Measurements

In order to establish the magnitude of a community odor problem it will be important to:

- identify the locations where odors have been and are perceived
- determine the frequency and duration of perceived odorous impacts
- measure the odor levels at the various locations where odors have been perceived (in terms of dilutions to detection thresholds, intensities or concentrations of key odorants)
- identify, whenever possible, any key odorants that can be related to perceived odors

Receptor Responses

Community reactions to existing ambient odors must be characterized through valid odor dosage-response correlations for a variety of conditions. This phase of the protocol would involve:

- analysis of past and current odor complaints where available
- analysis of the demographic nature of the affected community (age distributions, socio-economic activities and political forces are important)
- analysis of answers to an appropriate odor-survey questionnaire conducted in the affected community and a matching control area (to determine citizen prejudices and individual reactions to perceived odors)
- development of procedures for establishing the validity of current complaints

- development of an odor indexing or Odor Impact Model that will relate community odor detection, discrimination and complaint potential thresholds, as well as degrees of complaint, to the odor levels under consideration.

Successful verification that a suspected source (or sources) is or is not responsible for alleged odor problems in a community will require correlation of the data from on-site, off-site and receptor response studies.

Simultaneous sampling of source and ambient odors at times when complaints are being registered would help to settle controversial issues.

Fingerprinting, through identification of key odorants at on-site and off-site locations would, when coupled with reliable atmospheric dispersion modelling techniques, provide convincing technical evidence that a specific source is or is not responsible for community annoyance. Successful characterization of odor transport from source to receptors would require acquisition of:

- topographical data including locations of any wind screens
- meteorological factors including wind speed and direction as well as frequencies of wind directions
- ambient air parameters including temperatures, humidities and cloud covers
- locations of alternate odor sources such as sewer manholes, garbage bins and/or mobile sources.

Continued studies are still required if North American regulatory agencies are to develop legislation pertaining to odorous emissions. Lack of objective techniques for the measurement of source and ambient odor levels and community responses to odor episodes prevent control agencies from considering odorous industrial discharges to be more than "nuisance" problems.

NOMENCLATURE

$C_{(x,y,o)}$ = 1-hour average concentration at ground level point of impingement ($\mu g/m^3$)

Q = source emission rate of pollutant (g/s)

$N_{(x,y,o)}$ = C/C_{50} (dimensionless)

N_s = C_{source}/C_{50} (dimensionless)

C_{50} = 50% detection threshold concentration (ppm)

C_{source} = odor concentration at source (ppm)

C = downwind odor concentration (ppm)

u = wind velocity (m/s)

σ_y = crosswind plume standard deviation (m)

σ_z = vertical plume standard deviation (m)

H = effective plume height (m)

V_o = volumetric flow rate at source (m^3/s)

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*Final Reports Submitted to Ontario Ministry of the Environment

CHEMICAL SPECIATION OF AIRBORNE PARTICULATES

D.D. Burgess
McMaster University
Hamilton, Ontario

ABSTRACT:

Although neutron activation analysis provides high sensitivity and a low analytical blank, it does not allow determination of the chemical state of the elements detected. A method has been proposed whereby organic vapours are used to form volatile complexes of inorganic constituents of airborne particulates. These complexes could then be volatilised, removed from the sample and collected for neutron activation analysis. If the complex formation and volatilisation occur at different temperatures for different compounds of the same element, chemical speciation of that element should be possible.

The reactions of acetylacetone and its fluorinated derivatives with compounds of cadmium, iron and chromium are being studied to assess their possible utility for speciation. It has been found that although cadmium and its compounds react with the ligands tested, these reactions are too slow to be useful in analysis. The reactions of iron and chromium, however, are more rapid. The experimental methods adopted for this work will be described , results obtained to date will be discussed, and the course of future research on this topic will be outlined.

In order to prepare for future field studies in speciation, research in neutron activation analysis is also being carried out. A new method for the analysis of gamma-ray spectra has been developed and compared with other methods previously reported in the literature. The method developed in this laboratory proved to be as accurate as most other methods and more reliable in peak detec-

tion and in the treatment of multiplet peaks than several previously proposed techniques.

A new approach to optimisation of analytical procedures for activation analysis is under study. This new method, simplex optimisation by advance prediction (S.O.A.P.), involves calculation of spectra expected under given conditions and application of this information to simplex optimisation. The optimisation of single-element neutron activation analysis can now be achieved and multielement activation analysis is now being implemented . The progress made in these areas and the work yet to be done will be briefly described.

Introduction:

The proper assessment of the nature, behaviour and hazards of solid material present in the atmosphere requires methods for the determination of the composition of these materials. As airborne solids are found in low concentrations, highly sensitive techniques are needed for their analysis. These techniques should, ideally, provide data concerning not only the elemental composition of particulates but also the chemical forms of each element found.

Neutron activation analysis (NAA) is a highly sensitive analytical tool and has long been used for the elemental analysis of airborne particulates. NAA however, does not itself provide any information about the chemical form of the elements determined. This project addresses this problem.

It has been shown (1) that certain organic substances are capable of reacting with inorganic compounds to form volatile metal complexes. The approach to speciation adopted here employs this phenomenon to remove elements of interest from samples by volatilisation using organic vapours. The name reactive gas extraction has been given to this process.As different compounds may be expected to react with these vapours at different temperatures, selective volatilisation and hence speciation should be possible. At present, the chemistry of promising volatilisation reactions is being studied.

Once volatilisation of an element has been achieved, the volatile complex can be condensed and submitted to activation analysis. The use of only gaseous reagents should preserve the low analytical blank of NAA and the high sensitivity of NAA will permit the analysis of small quantities of material.

Experimental Methods:

Volatilisation experiments are carried out in a stream of carrier gas (nitrogen) mixed with ligand vapours. This gas stream is passed to a reaction tube which is held at a controlled temperature. The solid sample is placed in a pyrex cup provided with a thermocouple for temperature measurement. The gas, with volatilised complexes, is focussed by a capillary onto a collecting surface whose temperature is controlled to permit deposition of the complexes.

The progress of the volatilisation , in some experiments, will be monitored by means of radioactive samples and a radiation measurement instrument. It was found that a conventional tube furnace absorbed too much of the radiation. Accordingly, heating tapes are now used for temperature control. Separate temperature control is used for the ligand vapourisation, reaction, transfer and deposition zones. Figure 1. shows the experimental arrangement.

The ligands under study are moderately expensive. Therefore, to reduce losses, a cold trap and a peristaltic pump are used to recycle unreacted ligand. Figure 2. indicates the provisions for recycling.

Temperatures at the sample are measured by a digital thermocouple thermometer and recorded using a TRS-80 model I microcomputer. Radioactivity is monitored and recorded by a NaI(Tl) detector and a Canberra model 30 multichannel analyser operated in the multiscaling mode. The thermometer, multichannel analyser and the temperature program for the reaction zone of the volatilisation apparatus are controlled by a home-made electronic controller. Figure 3. gives the configuration used for data logging and temperature control.

It has been found that the volatile complexes and the ligands condense from the gas phase at similar temperatures. This makes collection of the complexes difficult and complicates radiotracer experiments. Reduction of the complexes to the metal and the free ligand using hydrogen has been reported for some complexes (2) and will be investigated as an alternative method for deposition of volatilised metals.

Ligands:

Several classes of compounds including β- diketones and dialkyldithiocarbamates have been employed for the creation of volatile metal complexes (3). The most important of these are the β- diketones and their fluorinated derivatives. The ligands investigated to date are 2,4-pentanedione, (AC) 1,1,1-trifluoro-2,4-pentanedione (TFA) and 1,1,1,5,5,5-hexafluoro-2,4,-pentanedione (HFA). These reagents have been purchased and used as received.

Results and Discussion:

Initial experiments concerned the volatilisation of cadmium. The volatilisation of cadmium and its compounds with the ligands listed above, however, has proved to be too slow to be analytically useful. Further work with cadmium will be carried out with sulphur analogues of β- diketones as these are known to form more stable complexes with cadmium than do the parent compounds.

Volatilisation of iron and chromium with β- diketones, on the other hand, has been more successful. Some observations of reactions of iron are given in Table 1. Ferric oxide may be confidently expected to react with HFA. The volatilisation reactions of iron and its compounds occur at convenient temperatures and evidently show sufficient temperature differences for speciation. Detailed radiotracer trials will be carried out to confirm this conclusion. A comprehensive study of the volatilisation reactions of β- diketones has now been undertaken.

The prospect of using a chemical reaction for deposition of metals from volatile complexes is attractive. The deposit would be easily manipulated for NAA. Further, if reaction temperatures are sufficiently lower than deposition temperatures, it should be possible to employ true chemical transport in a much simpler apparatus.

Auxiliary Studies:

When fully developed, the reactive gas extraction technique described above should permit multielement speciation analyses in which elements are distinguished by gamma-ray spectrometry during NAA. In preparation for this type of multielement analysis, research is also being conducted in NAA. These studies include methods for the analysis of gamma-ray spectra and computer aided design of procedures that use NAA.

A new approach to the estimation of the spectral baseline in gamma-ray spectra has been successfully developed (4) and compared to previous techniques. The new method compared favourably with older ones and permits greater flexibility in spectrum analysis.

Our research in automated method design is currently focussed on a new approach to the optimisation of conditions used in NAA. This method, called simplex optimisation by advance prediction (S.O.A.P.), uses spectra predicted (5) from the expected composition of the sample to provide values of a response function for use in simplex optimisation calculations (6,7). The adjustable parameters currently employed are irradiation, decay and counting times and sample size. Figure 4. shows the operation of the program.

The response function adopted for optimisation of single-element determinations is proportional to the detection limit (7) of the element of interest.

$$R= (2.71+4.65B^{0.5})/(A)$$

B baseline area under peak
A peak area

Figure 5. shows the response surface for the determination of arsenic in a hypothetical airborne particulate sample. The circle indicates the optimum found by the simplex program.

The simplex approach to optimisation of NAA has proven to be effective and very flexible. New response functions are easily implemented and limits to adjustable parameters and other quantities such as total count rate are easily achieved.

A comprehensive data set for airborne particulates is nearly complete and will be used to establish optimum conditions for the determination of important elements by NAA. A response function is being devised for the optimisation of multielement NAA and will be used to determine the conditions that permit the maximum information to be extracted from an airborne particulate sample.

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Table 1. Volatilisation with TFA

Sample	Temperature (at start of deposition) °C
Fe	200
$FeCl_3$	190
$FeCl_2.xH_2O$	180
Fe_2O_3	no reaction to 350
Cr	250

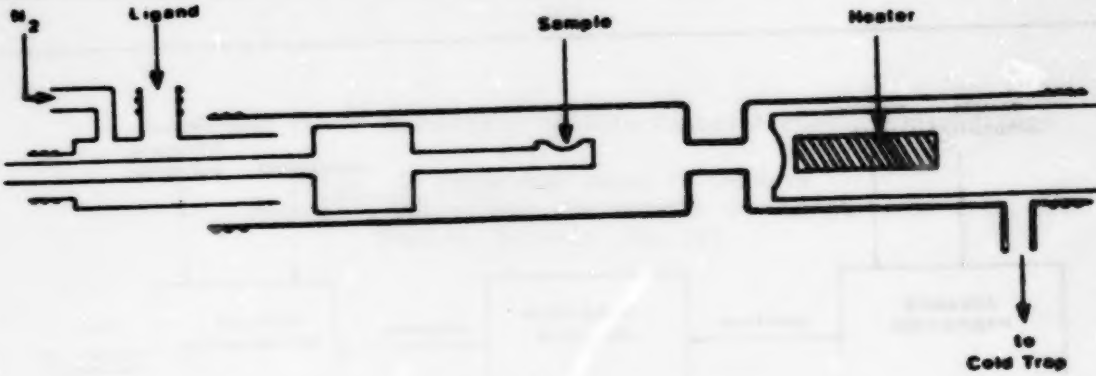

N2
Ligand
Sample
Heater
to Cold Trap

Figure 1. Volatilisation Apparatus

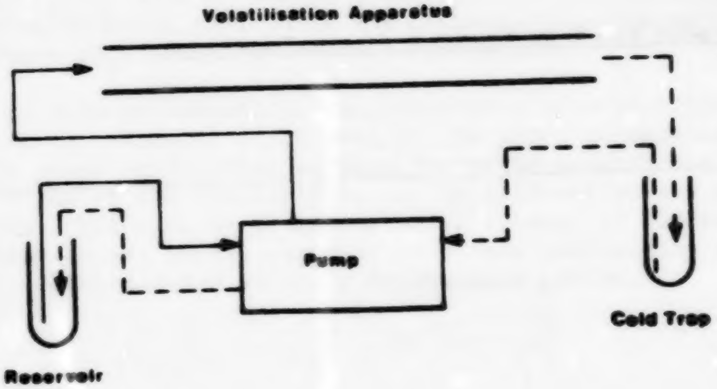

Volatilisation Apparatus
Pump
Cold Trap
Reservoir

Figure 2. Ligand Recycling

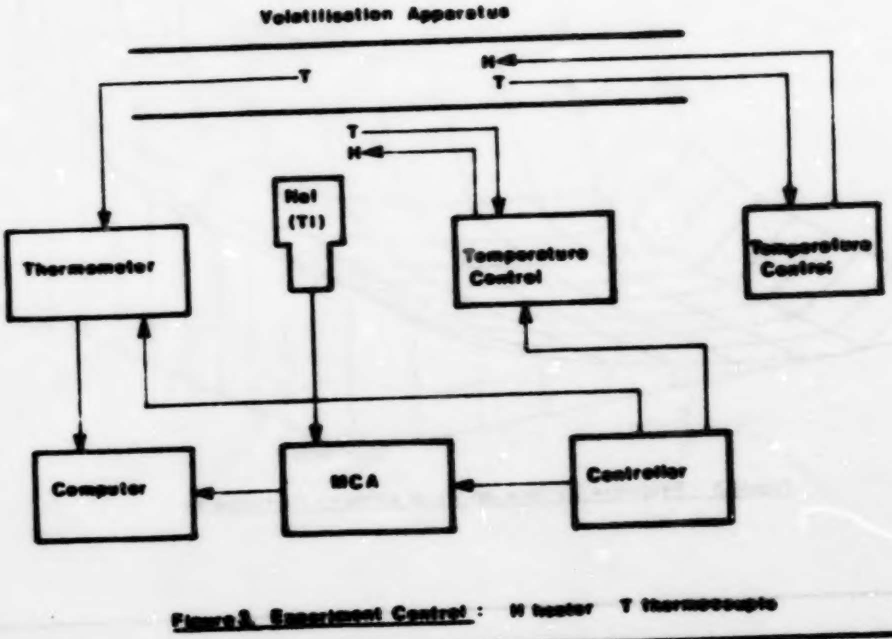

Volatilisation Apparatus
T
H
T
T
H
NaI (Tl)
Thermometer
Temperature Control
Temperature Control
Computer
MCA
Controller

Figure 3. Experiment Control : H heater T thermocouple

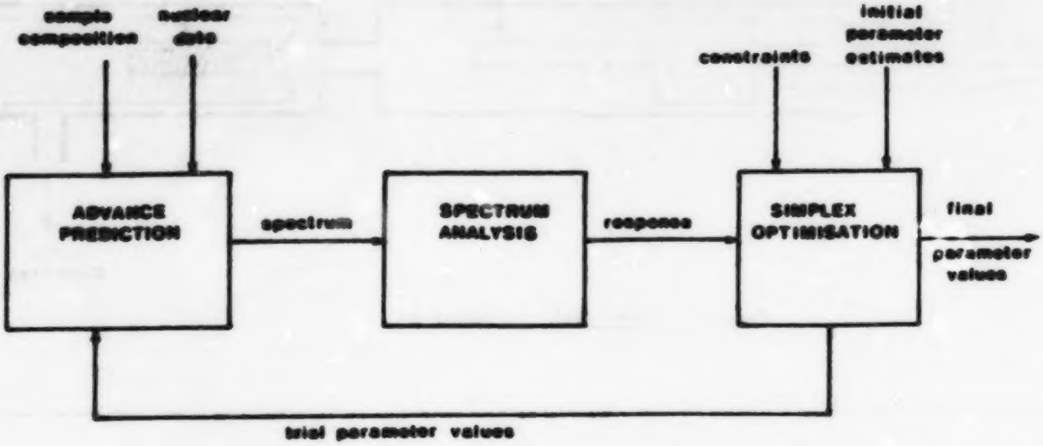

sample composition
nuclear data
constraints
initial parameter estimates
ADVANCE PREDICTION
spectrum
SPECTRUM ANALYSIS
response
SIMPLEX OPTIMISATION
final parameter values
trial parameter values

Figure4. Simplex Optimisation by Advance Prediction

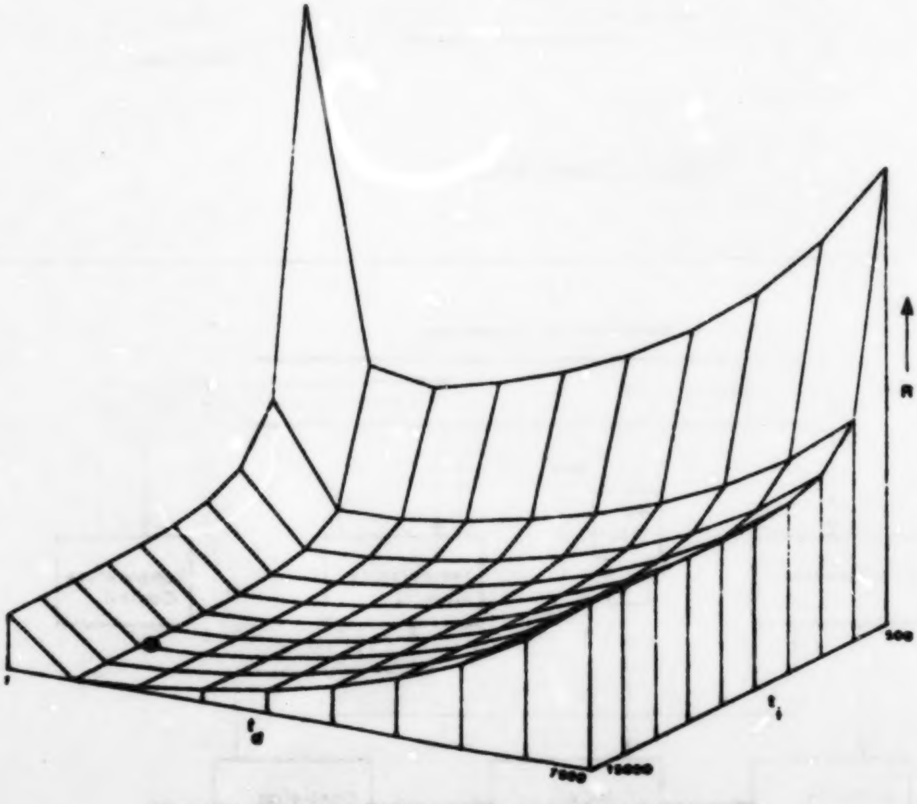


Figure 5. Response Surface for As in Airborne Particulates

Vapour Phase Photolysis of Chlorinated Aromatic Hydrocarbons

Nigel J. Bunce and James P. Landers
Chemistry Department, University of Guelph
Guelph, Ontario N1G 2W1

Abstract

Although there have been almost no published accounts of the vapour phase photochemistry of chlorinated aromatic hydrocarbons, the subject has potential environmental significance because typical aryl chloride pollutants are somewhat volatile. They therefore are potentially subject to solar degradation in the atmosphere. Our research is directed to studying the modes of photodecomposition of typical aromatic pollutants (chlorinated benzenes and biphenyls) under inert (N_2) and reactive (O_2) atmospheres.

In this presentation, the literature on aryl halide photochemistry is reviewed; since most of the previous studies have used solution phase conditions, we have attempted to indicate the extent to which any of the observations may be relevant to gas phase photochemistry. Finally, we present a brief account of the experimental procedures we are using, together with some preliminary results on product identification in the chlorobenzene series.

Chlorinated aromatic hydrocarbons are widespread and persistent pollutants. The EPA priority pollutant list (1978) mentions chlorobenzene, the three dichlorobenzenes, 1,2,4-trichlorobenzenes, hexachlorobenzene, and the polychlorinated biphenyls (PCB's) as examples of priority pollutants. DDT is an example of a chlorobenzene derivative (Figure 1).

In addition to the many studies of the biological chemistry of these chlorinated hydrocarbons, there have also been numerous photochemical studies. The justification for these photochemical studies has been that because these chlorinated hydrocarbons absorb light in the region of the solar spectrum (although very inefficiently) there may be a chance that sunlight assisted degradation is one of the routes by which these substances disappear from the environment.

To date, almost all the laboratory studies of these substances have been made using solutions of the chlorinated hydrocarbons in organic or in partly aqueous solvents. Any environmental aspect to this work therefore concentrates on the prospect that sunlight may decompose these substances in solution, for example, in bodies of water. Recently photochemical degradation using artificial light was used to destroy a "real sample" of one of the related class of compounds, the chlorinated dibenzodioxins, at a chemical manufacturing plant in the United States(1).

Pollutants such as the PCB's may be found in locations very remote from any industrial activity. They have, for example, been found in the snows of Antarctica$^{(2)}$. It follows that they must have reached this location by transport through the atmosphere. This is reasonable, given the relatively high volatility of many of these compounds.

Sample calculations

1. Chlorobenzene, b.p. 131°C = 404 K

   enthalpy of vaporization = 41.0 kJ mol^{-1}

   $\ln P_2/P_1 = \frac{\Delta H}{R}\left(\frac{T_2-T_1}{T_1T_2}\right)$

   $P_{273K} = 2.9\times10^{-3}$ atm $= 2.9\times10^{2}$ Pa

2. 2-Chlorobiphenyl, b.p. 274°C = 547 K

   ΔH_{vap} estimated from Trouton's Rule = 48,000 J mol^{-1}

   $\ln (P_{273}/P_{547}) = -10.6$

   $P_{273K} = 2.4\times10^{-5}$ atm = 2.4 Pa

3. 4-Chlorobiphenyl, b.p. 317°C = 590 K

   ΔH_{vap} estimated from Trouton's Rule = 52,000 J mol^{-1}

   $\ln (P_{273}/P_{590}) = -12.3$

   $P_{273K} = 4.6\times10^{-6}$ atm = 0.46 Pa

The research in which we are engaged is to understand the gas phase photochemistry of chlorinated aromatic compounds. We want to discover 1) the products of these reactions and 2) the detailed mechanisms by which the reactions occur so that 3) we can try to estimate whether the photolysis of these compounds by sunlight plays a significant role in their chemistry while they are present in the atmosphere. Because our research project commenced only on September 6, 1983, this account will be mainly devoted to what was already known about the photochemistry of these compounds.

Let us first make a digression concerning the concept of mechanism in a photochemical reaction. Besides the usual questions: "what intermediate species are formed en route to the final product?" and "how rapidly does each step in the pathway occur?", photochemical reactions pose two additional kinds of questions. These are: "which excited state of the starting material is involved?" and, "what is the 'quantum efficiency' of the reaction?" where the quantum efficiency is defined as the ratio below.

$$\text{Quantum efficiency, } \phi = \frac{\text{No. of molecules undergoing the process of interest}}{\text{Total no. of photons absorbed by the reactant}}$$

The "Jablonski Diagram" (Figure 2) summarises the main processes of interest. Singlet excited states differ from triplets in the relative alignments of the electron spins; for our purposes here we note only that triplet excited states tend to be quite a lot longer lived (microseconds) than singlet excited states (nanoseconds). The processes available to the excited states are:

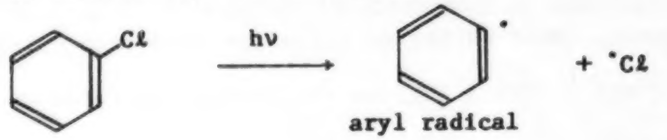
- return to the ground state with luminescence
- return to the ground state without luminescence
- singlet to triplet intersystem crossing (singlet only)
- chemical reaction.

Chemical reaction will proceed with more than negligible quantum yield only if the rates of the other competing processes are not too fast. Of course, in absolute terms, all chemical reactions of excited states must be very rapid, because of the speed of the competing processes which deactivate excited states. Consequently, photochemical reactions tend to occur only when they are energy releasing and have only small activation barriers.

Liquid Phase Studies

Chlorinated aromatic compounds have been investigated photochemically for 15-20 years. Essentially all these studies have been made in the liquid phase (usually in solution) and there is very little background of gas phase photochemistry upon which to draw. The primary photochemical act in most cases appears to be cleavage of the carbon-to-chlorine chemical bond (eq. 1); this is the weakest bond in the molecule.

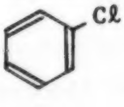
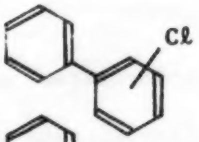
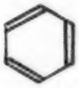
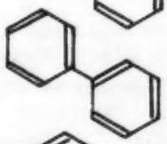
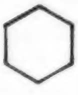
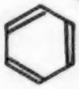
e.g.



[1]

The products of this initial cleavage are free radicals, which are very reactive. In solution, the final products depend upon what other compounds are available to undergo reaction with these radicals (Table 1).

Table 1: Products from photolysis of chlorobenzene in various solvents

Solvent	Structure	Product(s)	Structure
neat chlorobenzene(3)		chlorobiphenyls	
benzene(4)		biphenyl	
cyclohexane(5)		benzene hydrogen chloride	 + HCℓ

In benzene (or equivalently in neat liquid chlorobenzene) the favored reaction of the aryl radical is addition to the aromatic solvent, eventually giving a substitution product of the biphenyl series. Aliphatic solvents, exemplified by cyclohexane (but including alcohols, ethers, etc.) suffer hydrogen abstraction, and the aryl radical is reduced to benzene.

The photochemical efficiency of the reaction is strongly dependent on the energetics of eq. [1](6). The excited state involved is usually a triplet; if the energy of the state above the ground state is comparable with, or greater than, the C-Cℓ bond energy, dissociation occurs efficiently (high quantum yield). If the excited state energy is too low the reaction is very inefficient. Most estimates place the C-Cℓ bond energy in the region of 330 kJ mol^{-1}; Table 2 gives the triplet energies of some representative aryl chlorides.

Table 2. Triplet energies of aromatic systems

System		Triplet Energy, kJ mol^{-1}
Phenyl		350
Naphthyl		250
Biphenyl	not o-substituted	280
	o-substituted	330

Chlorinated benzenes and o-chlorinated biphenyls (Figure 3) tend to undergo dissociation with high efficiency ϕ 0.1-0.6. On the other hand, chlorinated napthalenes and non-o-chlorinated biphenyls are very resistant to photolysis, $\phi < 10^{-3}$. (For some of the chlorinated benzenes and naphthalenes, the reactions of photo-dimers complicate the picture, but these are not of concern at environmental concentrations). We may note also that aromatic bromides and, especially, iodides are more easily photodissociated than chlorides, because the bond to carbon is weaker.

A particular problem arises in the biphenyl series in that the o-chloro compounds are much more photolabile than those lacking o-substituents. This is a steric effect. The ground state of biphenyl is best described as having a single bond between the two benzene rings (Figure 4a); a comparable description of the excited state has the rings coplanar (Figure 4b). Substitutents larger than hydrogen cause steric compression, which is relieved when the substituent (chlorine) departs. Additionally, the energy content of the o-excited state is higher, again because of steric compression (Table 2). This point is discussed in more detail in reference 7.

Vapour phase studies

Only one report of the gas phase photochemistry of chlorobenzene has appeared(8). In that work, Ichimura and Mori photolysed chlorobenzene in an atmosphere of ethane. The products observed were benzene, HCℓ, and butane. It is clear that under these conditions the reaction is exactly analogous to that observed in solution with, for example, cyclohexane. The products are explained by Scheme 1.

Scheme 1

$$PhC\ell^{*} \longrightarrow Ph^{\bullet} + C\ell^{\bullet}$$

$$Ph^{\bullet} + C_2H_6 \longrightarrow PhH + C_2H_5^{\bullet}$$

$$C\ell^{\bullet} + C_2H_6 \longrightarrow HC\ell + C_2H_5^{\bullet}$$

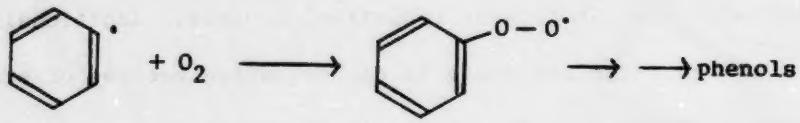
$$2C_2H_5^{\bullet} \longrightarrow C_4H_{10}$$

For the present purposes, what is significant is that the quantum yield of reaction (0.4) is very similar to that found in solution. In solution, there could be cage recombination (eq. 2) which would make the observed quantum yield of decomposition smaller than the primary quantum yield of decomposition.

$$(C\ell^{\bullet} + Ph^{\bullet}) \longrightarrow PhC\ell \qquad [2]$$

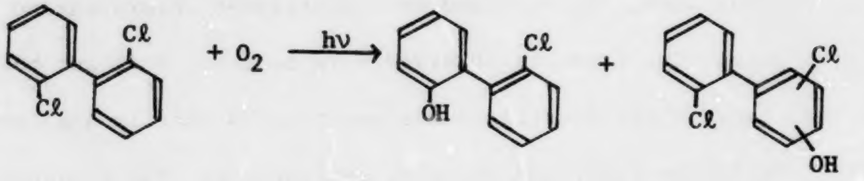
The similarity of quantum yields suggests that this is not a problem in practice; more importantly, it suggests a primary $\phi_{decomp.}$ of ca 0.4 should be observed in any gas phase reaction of chlorobenzene. Therefore in ordinary air, we would expect that the first formed radicals in scheme 1 should disappear by reaction with oxygen, eq. 3.

e.g.



[3]

The expectation of eq. 3 appears to be fulfilled in the chlorobiphenyl series, where the only published gas phase study, that of Hustert and Korte(9), indeed gave a phenolic product eq. 4.



[4]

In this study, 2,2′-dichlorobiphenyl underwent photodecomposition to afford two products. In one of them, hydroxyl replaced one of the chlorine substituents, presumably by some reaction analogous to eq. 3. The other product was incompletely identified, but contained a hydroxyl function additional to the two chlorines. We would expect, again by analogy with the solution phase studies, that PCB′s containing ortho chlorines should be much more photolabile in the gas phase than those without.

In further work, Prof. Korte and his students have concentrated extensively on the "photomineralization" of PCB′s and other substances. Photomineralization is the term used by this research group for the complete photodegradation (to HCℓ and CO_2) of the pollutants adsorbed on particles of silica gel. Such reactions are intended to model the fate of the pollutant adsorbed on particulate matter in the atmosphere. They are thus complementary to our studies in which the pollutants are photolysed in the gas phase.

Among chloro compounds which are not aromatic, a research group at Dow Chemical Co. has studied the simulated at atmospheric photodecomposition of simple aliphatic chlorides such as chlorinated ethenes and ethylenes(10). These reactions were carried out with 10 ppm of the organic compound in the presence of ca. 5 ppm of NO to simulate a polluted atmosphere. Under conditions controlled to imitate bright sunlight, all the mono-, di-, and trichloroethylenes had half lives for decomposition $\tau_{1/2}$ of 5-12 h. Chlorobenzene is mentioned briefly in this paper, its $\tau_{1/2}$ was ca. 9 h. No products were identified.

The present work

The reaction chamber which we have had made consists of a 12 litre flask equipped with three ground glass joints. The largest of these (60/50) accommodates an inversion well apparatus which projects inside the flask. The lamp, a 450 W "Hanovia" medium pressure mercury arc, rests in the centre of the immersion well and is cooled by a flow of water. We have available both quartz and Pyrex inversion wells; the former transmits all radiation λ >220 nm, the latter only λ >290 nm. Although the Pyrex equipment more closely simulates sunlight, it is convenient to use the quartz for speedier reaction times in our exploratory experiments.

The remaining inlets to the flask are used to admit and remove gas. The chloroaromatic compound is placed in a reservoir, which may be heated if necessary. Gas (N_2 or O_2) is passed slowly through the reservoir, and entrains some of the vapour of the chlorocompound. After the gases leave the reaction chamber, they are condensed at -78°C in a dry ice cold trap. After reaction the contents of the trap and the reaction flask are separately dissolved in a suitable solvent, and analysed by vapour phase chromotography and GC-mass spectrometry.

Our first experiments have been carried out using chlorobenzene with the quartz equipment and a nitrogen (inert) atmosphere. Under static conditions (no flow, the chlorobenzene placed directly in the reaction flask), most of the product is an intractable polymer. At low conversions we see the expected products of bimolecular reactions e.g. monochlorobiphenyls (compare Table 1). Under conditions of flow, at least two unimolecular products (similar volatility to starting material) are formed. These compounds appear to decompose thermally on standing and are not yet identified. We speculate that they may be "valence isomers" of the starting material. Similar valence isomers have been detected previously upon photolysis of benzene and its methyl derivatives.

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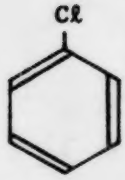
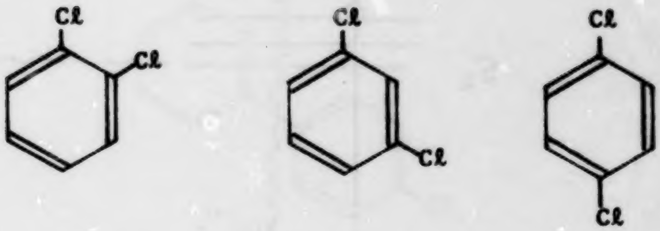
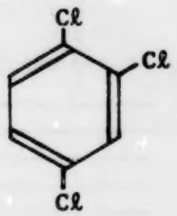


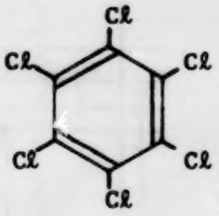
chlorobenzene



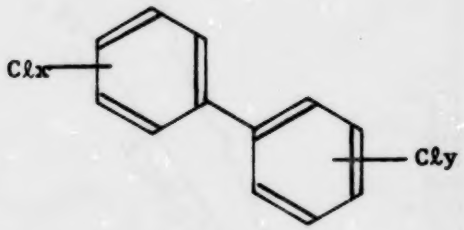
o- , *m*- , and *p*-dichlorobenzenes



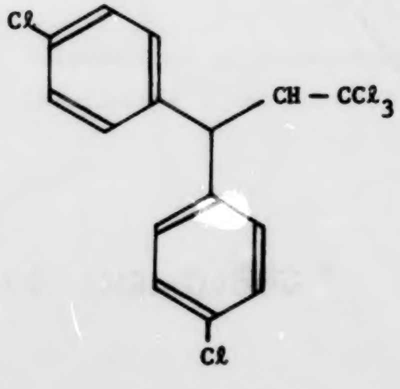
1,2,4-trichlorobenzene



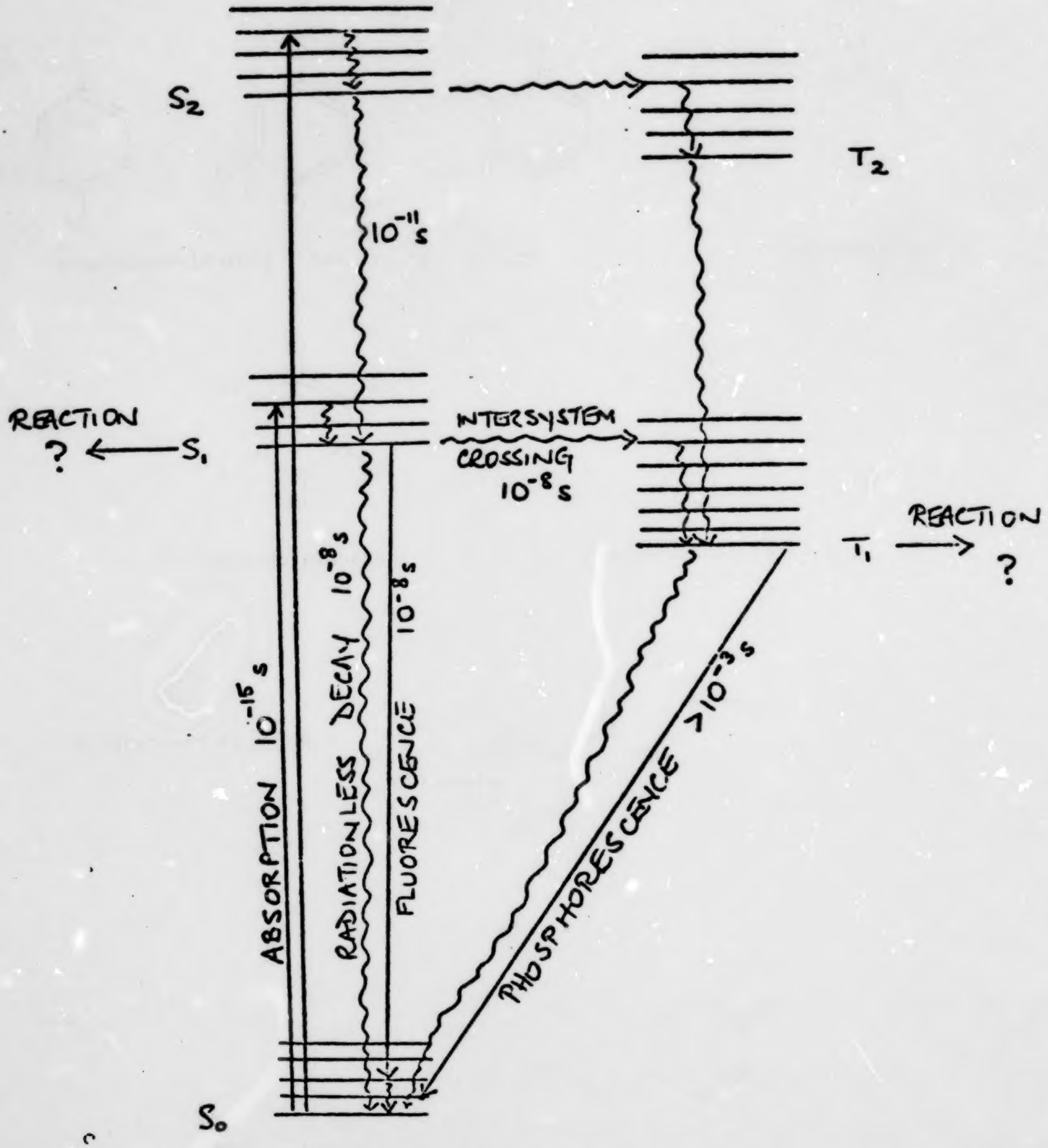
hexachlorobenzene



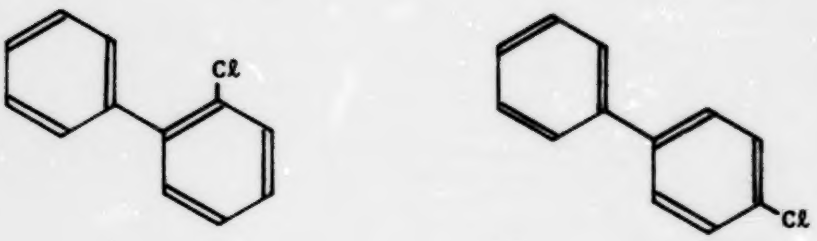
x and y in the range 1-5



p,p' — DDT


S₂
T₂
10⁻¹¹ s
REACTION
?
S₁
INTERSYSTEM
CROSSING
10⁻⁸ s
REACTION
T₁
?
ABSORPTION 10⁻¹⁵ s
RADIATIONLESS DECAY 10⁻⁸ s
FLUORESCENCE 10⁻⁸ s
PHOSPHORESCENCE >10⁻³ s
S₀

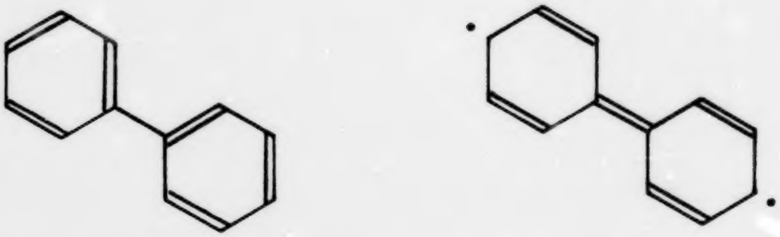
"JABLONSKI DIAGRAM"



o-chlorobiphenyl

p-chlorobiphenyl

Figure 3: Examples of *o*- and non-*o*-chlorobiphenyls



(a)

(b)

Figure 4: Valence bond representations of (a) ground state (b) first excited state of biphenyl

POOR

QUALITY

ORIGINAL